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**Exendin analogues, processes for their preparation and medicaments containing them**

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<p>(54) Title: EXENDIN ANALOGUES, PROCESSES FOR THEIR PREPARATION AND MEDICAMENTS CONTAINING THEM</p> <p>(54) Bezeichnung: EXENDIN-ANALOGA, VERFAHREN ZU DEREN HERSTELLUNG UND DIESE ENTHALTENDE ARZNEIMITTEL</p> <div style="margin-top: 20px;"> <p>SEQ ID NO: 1</p> <table style="width: 100%; border-collapse: collapse;"> <tr> <td style="text-align: center;">1</td> <td style="text-align: center;">5</td> <td style="text-align: center;">10</td> <td style="text-align: center;">15</td> <td></td> </tr> <tr> <td>H</td><td>S</td><td>D</td><td>G</td><td>T</td><td>F</td><td>T</td><td>S</td><td>D</td><td>L</td><td>S</td><td>K</td><td>Q</td><td>M</td><td>E</td><td>E</td><td>E</td><td>A</td><td>V</td> </tr> <tr> <td>20</td><td></td><td></td><td></td><td></td><td>25</td><td></td><td></td><td></td><td>30</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td> </tr> <tr> <td>R</td><td>L</td><td>F</td><td>I</td><td>E</td><td>W</td><td>L</td><td>K</td><td>N</td><td>G</td><td>X</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td> </tr> </table> </div> <div style="margin-top: 20px;"> <p>SEQ ID NO: 2</p> <table style="width: 100%; border-collapse: collapse;"> <tr> <td style="text-align: center;">1</td> <td style="text-align: center;">5</td> <td style="text-align: center;">10</td> <td style="text-align: center;">15</td> <td></td> </tr> <tr> <td>H</td><td>G</td><td>E</td><td>G</td><td>T</td><td>F</td><td>T</td><td>S</td><td>D</td><td>L</td><td>S</td><td>K</td><td>Q</td><td>M</td><td>E</td><td>E</td><td>E</td><td>A</td><td>V</td> </tr> <tr> <td>20</td><td></td><td></td><td></td><td></td><td>25</td><td></td><td></td><td></td><td>30</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td> </tr> <tr> <td>R</td><td>L</td><td>F</td><td>I</td><td>E</td><td>W</td><td>L</td><td>K</td><td>N</td><td>G</td><td>X</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td> </tr> </table> </div> <p>(57) Abstract</p> <p>The invention concerns novel exendin analogues which can be used in the treatment of diabetes mellitus. The invention also concerns processes for preparing these substances and medicaments containing them. The exendin analogues are derived from SEQ ID NO: 1 (I) or SEQ ID NO: 2 (II).</p> <p>(57) Zusammenfassung</p> <p>Diese Erfindung betrifft neue Exendin-Analoga, welche bei der Therapie des Diabetes mellitus eingesetzt werden können, Verfahren zu deren Herstellung und diese enthaltende Arzneimittel. Die Exendin-Analoga leiten sich von SEQ ID NO: 1 (I) oder SEQ ID NO: 2 (II) ab.</p>			1	5	10	15		H	S	D	G	T	F	T	S	D	L	S	K	Q	M	E	E	E	A	V	20					25				30										R	L	F	I	E	W	L	K	N	G	X									1	5	10	15		H	G	E	G	T	F	T	S	D	L	S	K	Q	M	E	E	E	A	V	20					25				30										R	L	F	I	E	W	L	K	N	G	X								
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**Exendin analogues, processes for their production and pharmaceutical preparations containing them**

This invention concerns new exendin analogues which can be used in the therapy of diabetes mellitus, processes for their production and pharmaceutical preparations containing them.

**Background of the invention**

A functional connection between the small intestine and exocrine pancreas was proven in the sixties after it became possible to accurately determine insulin in plasma. The insulin response after oral glucose administration is much more stronger than after intravenous glucose administration even if identical plasma levels of glucose are reached. This "incretin effect" is explained by the functional combination of the entero-insular axis. Intestinal hormones are responsible for this effect which are released from the small intestine after meals, circulate in the plasma at increases measurable levels and amplify glucose-induced insulin release. In addition to the classical incretin hormone gastric inhibitory polypeptide I (GIP), nowadays glucagon-like peptide 1 (GLP-1) is nowadays of primary interest. In a relatively short time GLP-1 has matured from being the physiologically most interesting incretin hormone candidate to a potential alternative for the treatment of diabetes mellitus type II. The present invention describes new substances which imitate the effect of the naturally occurring GLP-1 molecule. These new substances are characterized by an increased stability while maintaining the efficacy.



### Anti-diabetogenic action

Infusion and subcutaneous injection of GLP-1 cause a considerable increase of insulin secretion and an inhibition of glucagon release in patients with diabetes mellitus type II (Gutniak, M. (1992); Kreymann, B. (1987); Nathan, D.M. (1992); Nauck, M.A. (1993a & b)). Both are of therapeutic interest and are involved in the blood sugar lowering effect of GLP-1: insulin promotes glucose uptake by its target tissue and inhibits gluconeogenesis. Furthermore GLP-1 analogues would be expected to increase glucose uptake in the periphery. The inhibition of glucagon secretion must be regarded as an indirect GLP-1 effect since glucagon-producing A cells express no GLP-1 receptors (Komatsu, R. (1989)). On the contrary, the increased insulin and somatostatin release appear to be the decisive factor. Both hormones are known as inhibitors of glucagon release.

Two molecular mechanisms certainly contribute to the GLP-1-induced insulin release in diabetes mellitus type II. In addition to directly amplifying the glucose-induced insulin release, GLP-1 sensitises a subgroup of B cells towards the key stimulus "glucose" (Fehmann, H.C. (1991)) and possibly also towards further stimuli so that overall more B cells secrete insulin. This priming effect is the most likely explanation for the fact that GLP-1 leads to a prolonged release of insulin despite its relatively short plasma half-life.

This effect depends on increased glucose levels (> 108 mg/dl) (Göke, R. (1993a)). It distinguishes GLP-1 fundamentally from the sulfonylureas which influence insulin secretion independently of the plasma level of glucose. If the glucose value decreases below 108 mg/dl, the insulin secretion dries up even with an intravenous



infusion of GLP-1. Hence hypoglycaemias would be hardly expected when GLP-1 is used therapeutically. In fact they have also not been described in the previous clinical studies. However, the pharmacokinetic properties of GLP-1 are problematic. The duration of action is limited due to its very short half-life.

From a therapeutic point of view the synthesis of stable and strongly effective GLP-1 peptide analogues is in any case desirable. Peptide analogues have now been synthesized based on the molecule exendin that was originally isolated from the venom of lizards with the aim of developing improved therapeutic agents that are stable towards degradation with an increased duration of action for the treatment of diabetes mellitus. These peptides have the same pharmacological effect as GLP-1, but surprisingly have a considerably longer half-life.

The new peptide sequences described as the subject matter of the invention have an effect on insulin synthesis and insulin release and an action on the insulin effect especially the uptake of glucose into the target tissues, muscle and fat tissue as well as emptying of the stomach.

#### **Subject matter of the invention**

The present invention is based on the sequence of exendin-3 and exendin-4, peptides which were isolated from the secretory product of *Heloderma horridum* or *Heloderma suspectum* (Eng. J. et al. (1990, 1992)). The amino acid sequence and effect of the two peptides on the pancreas has already been published by several authors (Eng. J. et al. (1990); Raufman, J.P. (1992); Göke, R. (1993b); Thorens, B. (1993)). The subject



matter of this invention are new truncated exendin fragments which comprise the amino acid sequences of exendin-3-(1-30) or exendin-4-(1-30) in which the C-terminal end of these sequences can be shortened by up to 3 amino acids, preferably by at most 1 amino acid, and the N-terminal end can be shortened by up to 2 and preferably at most 1 amino acid. Surprisingly these exendin fragments are biologically active although the amino acid sequence is shortened. Shortened amino acid sequences are more economical to produce than relatively longer sequences. Fragments with the following sequences are particularly preferred; especially peptide fragments that are based on exendin-3-(1-30) (SEQ ID No. 1).

10 The present invention provides a peptide, wherein it has the sequence 1 or 2

SEQ ID NO: 1 based on exendin-3

15  
 1                      5                      10                      15  
 H S D G T F T S D L S K Q M E E E A V  
 20                      25                      30  
 R L F I E W L K N G X<sub>1</sub>

SEQ ID NO: 2 based on exendin-4

20  
 1                      5                      10                      15  
 H G E G T F T S D L S K Q M E E E A V  
 20                      25                      30  
 R L F I E W L K N G X<sub>1</sub>

25 in which X denotes one of the 20 proteinogenic amino acids from Table 1 apart from glycine or a non-proteinogenic, the amino acids in position 1, 2, 28, 29 or 30 can be part of the sequence depending on the desired chain length and the N-terminus is represented by NR<sub>1</sub>R<sub>2</sub> in which

R<sub>1</sub> denotes hydrogen, acetyl, trifluoroacetyl, adamantyl, Fmoc, Z, Boc, Alloc, C<sub>1</sub>-C<sub>6</sub> alkyl, as hereinbefore defined, C<sub>2</sub>-C<sub>8</sub> alkenyl or C<sub>7</sub>-C<sub>9</sub> aralkyl,  
 R<sub>2</sub> denotes hydrogen, acetyl, trifluoroacetyl, adamantyl, Fmoc, Z, Boc, Alloc,



C<sub>4</sub>-C<sub>6</sub> alkyl, as hereinbefore defined,  
C<sub>2</sub>-C<sub>8</sub> alkenyl or C<sub>7</sub>-C<sub>9</sub> aralkyl,  
and the C-terminus is represented by COR<sub>3</sub> in which R<sub>3</sub> equals OR<sub>4</sub> or NR<sub>4</sub>R<sub>5</sub>  
in which R<sub>4</sub> equals hydrogen or C<sub>1</sub>-C<sub>6</sub> alkyl, as hereinbefore defined,  
5 in which R<sub>5</sub> equals hydrogen or C<sub>1</sub>-C<sub>6</sub> alkyl, as hereinbefore defined,  
as well as physiologically tolerated salts and esters thereof.

The invention also provides a pharmaceutical preparation containing at least one peptide of  
sequence 1 or 2 in addition to conventional carriers and auxiliary substances.

10

In yet another aspect there is provided use of at least one peptide of sequence 1 or 2 for the  
production of a pharmaceutical composition for the treatment of diabetes and a method of  
treating diabetes comprising the administration of at least one peptide of sequence 1 or 2 to  
a subject in need thereof.

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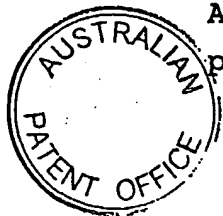
Exendin and exendin analogues with a chain length of 1-27 are preferred and especially those with a chain length of 1-30.

The carboxyl group  $\text{COR}_3$  of the amino acid at the C-terminal end can be present in a free form ( $\text{R}_3 = \text{OH}$ ) or in the form of a physiologically tolerated alkaline or alkaline earth salt such as e.g. a sodium, potassium or calcium salt. The carboxyl group can also be esterified with primary, secondary or tertiary alcohols such as e.g. methanol, branched or unbranched  $\text{C}_1\text{-C}_6$ -alkyl alcohols, in particular ethyl alcohol or tert. butanol. The carboxyl group can, however, also be amidated with primary or secondary amines such as ammonia, branched or unbranched  $\text{C}_1\text{-C}_6$  alkylamines or  $\text{C}_1\text{-C}_6$  di-alkylamines, in particular methylamine or dimethylamine.

The amino group of the amino acid  $\text{NR}_1\text{R}_2$  at the N-terminal end can be present in a free form ( $\text{R}_1, \text{R}_2 = \text{H}$ ) or in the form of a physiologically tolerated salt such as e.g. a chloride or acetate. The amino group can also be acetylated with acids so that  $\text{R}_1 = \text{H}$  and  $\text{R}_2 = \text{acetyl}$ , trifluoroacetyl, adamantyl or be present in a form protected by conventional amino protecting groups of peptide chemistry such as e.g. Fmoc, Z, Boc, Alloc or be N-alkylated in which  $\text{R}_1$  and/or  $\text{R}_2 = \text{C}_1\text{-C}_6$  alkyl or  $\text{C}_2\text{-C}_8$  alkenyl or  $\text{C}_7\text{-C}_9$  aralkyl.

Alkyl residues are understood as straight-chained, branched or optionally ring-shaped alkyl residues, preferably methyl, ethyl, isopropyl and cyclohexyl.

All exendin fragments can be present as completely or partially protected derivatives.



A further subject matter of this invention are exendin fragments with the above-mentioned properties and sequence lengths in which at least one but at most eleven of the modifications listed under (a) to (p) have been additionally carried out. Exendin fragments are preferred which have at most nine and particularly preferably those which have at most five of the modifications listed under (a) to (p).

- (a) The  $\alpha$ -amino acid in position 1 is D-His, Ala, D-Ala, Gly, Lys oder D-Lys of which Ala, Gly or Lys are particularly preferred; or
- (b) the  $\alpha$ -amino acid in position 2 is Ser, D-Ser, Thr, D-Thr, Gly, Ala, D-Ala, Ile, D-Ile, Val, D-Val, Leu or D-Leu, preferably Ser, Thr, Gly, Ala, Val, Ile or Leu; or
- (c) the  $\alpha$ -amino acid in position 3 is Glu, D-Glu, Asp, D-Asp, Ala oder D-Ala, preferably Glu, Asp or Ala; or
- (d) the amino acid in position 4 is Ala, D-Ala oder  $\beta$ -Ala, preferably Ala; or
- (e) the  $\alpha$ -amino acid in position 5 is Ser, Tyr or Ala; or
- (f) the  $\alpha$ -amino acid in position 6 is Ala, Ile, Val, Leu, Cha or Tyr, preferably Ala, Ile, Val, Leu or Tyr; or
- (g) the  $\alpha$ -amino acid in position 7 is Ala, D-Ala, Tyr, D-Tyr, Ser, D-Ser or D-Thr, preferably Ala, Tyr or Ser; or
- (h) the  $\alpha$ -amino acid in position 8 is Ala, Tyr or Thr; or
- (i) the  $\alpha$ -amino acid in position 9 is Ala, D-Ala, Glu, D-Glu or D-Asp, preferably Ala or Glu; or
- (j) the amino acids in position 10, 11, 12, 15, 16, 17, 18, 19, 20, 21, 24, 28, 29 are independently of one



- another a proteinogenic or non-proteinogenic D- or L-amino acid, preferably a proteinogenic L-amino acid; or
- (k) the  $\alpha$ -amino acid in position 13 is a neutral L-amino acid, preferably a neutral proteinogenic L-amino acid; or
  - (l) the  $\alpha$ -amino acid in position 14 is, for the purposes of stabilization, replaced by a neutral L- or D-amino acid, apart from L-leucine, preferably by Nle, D-Nle, Ala, D-Ala, Ile, D-Ile, Val or D-Val, wherein Ile, Val or Ala are particularly preferred; or
  - (m) the  $\alpha$ -amino acid in position 22 is D-Phe, Tyr, D-Tyr, Leu, D-Leu, Val, D-Val, L-Cha, D-Cha,  $\beta$ -1-Nal,  $\beta$ -2-Nal or  $\beta$ -1-D-Nal, wherein Tyr, Leu or Val are preferred; or
  - (n) the  $\alpha$ -amino acid in position 23 is Leu, D-Leu, D-Ile, Val, D-Val, L-Cha, D-Cha, Tyr, D-Tyr, Phe or D-Phe, wherein Leu, Val, Tyr or Phe are preferred; or
  - (o) the  $\alpha$ -amino acid in position 25, 26 or 27 is a neutral L- or D-amino acid, preferably a neutral, proteinogenic L-amino acid; or
  - (p) the  $\alpha$ -amino acid in position 30 is a proteinogenic or non-proteinogenic D- or L-amino acid apart from glycine, preferably Arg, D-Arg, Tyr or D-Tyr, Arg or Tyr are particularly preferred.

Among the new exendin fragments, those are particularly preferred which contain the amino acid leucine at position 10 and/or the amino acid valine at position 19, the amino acid isoleucine or alanine instead of methionine at position 14 and arginine at position 30 in addition to the already mentioned properties and sequence lengths. Those modifications of exendin fragments are also particularly preferred in which, in



addition to the said particularly preferred amino acids at positions 10, 14, 19 and 30, one of the 20 known proteinogenic L-amino acids is located at position 2.

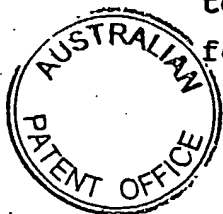
Preferred exendin analogues have a substitution at position 3 or 14, particularly preferably at position 2 and especially preferably the exendin analogues only contain proteinogenic amino acids.

In addition to new shortened and stabilized exendin-3 and exendin-4 analogues, the invention also concerns processes for producing these analogues in which the analogue are prepared in a solid phase synthesis from protected amino acids contained in the analogues which are coupled in sequence and correspond to the amino acids in the analogues and which are optionally supplemented with corresponding amino acids which do not occur in the natural exendin peptides.

The glycine at position 30 of the exendin-3 or exendin-4 sequence was substituted by another proteogenic or non-proteogenic amino acid in order to avoid diketopiperazine formation during the synthesis after cleavage of the amino terminal protective group.

The exendin-(1-30) analogues and fragments are advantageous compared to the exendins-1-(1-39) since the shorter sequences of these analogues enable a more simple synthesis in higher yields.

The abbreviations and definitions of the amino acids that are used were recommended in Pure Appl. Chem. 31, 639-45 (1972) and ibid. 40, 277-90 (1974) and correspond to the PCT rules (WIPO standard st. 23: Recommendation for the Presentation of Nucleotide and Amino Acid



Sequences in Patent Applications and in Published Patent Documents). The one and three letter codes are as follows:

Amino acid abbreviations

Amino acid	three letter code	one letter code
alanine	Ala	A
arginine	Arg	R
asparagine	Asn	N
aspartic acid	Asp	D
cysteine	Cys	C
glutamine	Gln	Q
glutamic acid	Glu	E
glycine	Gly	G
histidine	His	H
isoleucine	Ile	I
leucine	Leu	L
lysine	Lys	K
methionine	Met	M
phenylalanine	Phe	F
proline	Pro	P
serine	Ser	S
threonine	Thr	T
tryptophan	Trp	W
tyrosine	Tyr	Y
valine	Val	V
other amino acids	Xaa	X

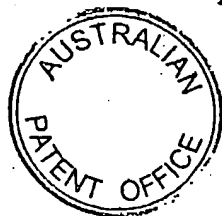
The abbreviations represent L-amino acids if not specified otherwise such as D- or D,L-. The D-amino



acids are written in small letters in the one letter code. Certain natural as well as non-natural amino acids are achiral e.g. glycine. In the representation the N-terminal end of all peptides is on the left and the C-terminal end is on the right.

Examples of non-proteinogenic amino acids are given in the following list together with their abbreviations:

$\beta$ -alanine	$\beta$ -Ala
o-aminobenzoic acid	Oab
m-aminobenzoic acid	Mab
p-aminobenzoic acid	Pab
m-aminomethylbenzoic acid	Amb
$\omega$ -aminohexanoic acid	Ahx
$\omega$ -aminoheptanoic acid	Ahp
$\omega$ -aminooctanoic acid	Aoc
$\omega$ -aminodecanoic acid	Ade
$\omega$ -aminotetradecanoic acid	Atd
citrulline	Cit
cyclohexylalanine	Cha
$\alpha,\gamma$ -diaminobutyric acid	Dab
$\alpha,\beta$ -diaminopropionic acid	Dap
methionine sulfoxide	Met(O)
C <sup><math>\alpha</math></sup> -methyl-alanine	Aib
N-methyl-glycine (sarcosine)	Sar
naphthylalanine	Nal



norleucine	Nle
ornithine	Orn
1,2,3,4-tetrahydroisoquinoline- 3-carboxylic acid	Tic

All amino acids can be divided into the following three main classes according to their physical-chemical properties:

Acidic: The amino acid releases a proton in aqueous solution and at physiological pH and consequently carries a negative charge.

Basic: The amino acid accepts a proton in aqueous solution and at physiological pH and consequently carries a positive charge.

Neutral: The amino acid is in an uncharged state in aqueous solution and at physiological pH.

The definition "carries a positive/negative charge" or "is in an uncharged state" only applies when on statistical average a significant number of a class of amino acids (at least 25 %) are in the said state.

In addition to the 20 so-called proteinogenic amino acids whose incorporation into proteins is controlled by the information of the genetic code, non-proteinogenic amino acids can also be incorporated into peptide sequences by the described synthesis process. A list of the proteinogenic amino acids and their classification into the above-mentioned three classes is given in Table 1. Non-proteinogenic amino acids are not genetically coded. Examples of non-proteinogenic amino acids and their classification into acidic, basic or neutral amino acids is given in Table 1.



Table 1

	proteinogenic	non-proteinogenic
acidic	Asp, Glu	
basic	Arg, His, Lys	Dab, Dap, Orn
neutral	Ala, Asn, Cys, Gln, Gly, Ile, Leu, Met, Phe, Pro, Ser, Thr, Trp, Tyr, Val	$\beta$ -Ala, Aib, Cit, Cha, Oab, Mab, Pab, Amb, Ahx, Ahp, Aoc, Ade, Atd, Nal, Nle, Sar, Tic

The exendin analogues which are a subject matter of the invention have advantageous therapeutic properties. Hence they lead to a stimulation of insulin release from the endocrine pancreas, to an increase of insulin biosynthesis and to increased peripheral glucose utilization. Since these effects can only be observed when the blood sugar levels are at the same time increased, a hypoglycaemia would not be expected to occur after their administration. Furthermore the exendin analogues inhibit glucagon release from the endocrine pancreas and lead to a decrease of gluconeogenesis. In non-insulin dependent diabetes mellitus (NIDDM) the exendin analogues result in a considerable improvement of the metabolic situation. In particular the glucose uptake in muscle and fat tissue is increased independently of the insulin secretory effect. Due to the inhibitory effect on glucagon release, it is also appropriate to administer the exendin analogues in insulin dependent diabetes mellitus. Compared to glucagon-like peptide 1 (GLP-1) and the known exendin-3 and exendin-4 sequences, the exendin analogues according to the invention surprisingly have a higher efficacy in the various test systems so that they are more suitable for a therapeutic application than GLP-1, exendin-3 or exendin-4. The advantages of the new exendin analogues are in particular as follows: higher stability towards





degradation and metabolism, longer duration of action, effectiveness at lower doses. Analogues based on exendin-3 are particularly preferred which exhibit particularly long durations of action or effectiveness at particularly low doses.

Solid phase and liquid phase synthesis is a conventional process for synthesizing peptides. In order to optimize the process for the synthesis of a particular product with regard to the purity of the crude product and yield, it is necessary that the process parameters and the materials that are used, for example the support material, the reagents which should make groups react, the materials for blocking the groups which should not react or the reagents which cleave blocking materials are adapted to the product to be synthesized, to the intermediate products to be synthesized and the starting materials. This adaptation is not simple with regard to the interdependency of the many process parameters.

Pharmaceutical preparations which contain the peptides according to the invention individually or together as an active substance in addition to conventional auxiliary substances and additives are preferably administered parenterally (subcutaneously, intramuscularly or intravenously). However, all other common forms of administration such as oral, rectal, buccal (including sublingual), pulmonary, transdermal, iontophoretic, vaginal and intranasal administration come into consideration. The drug has an insulin-regulating effect thereby promoting in an advantageous manner the compensation of the blood sugar level. It is advantageous for the use of the drug when blood levels between 20 and 50 pmol/l are attained. Infusion rates of 0.4 - 1.2 pmol/kg/min are necessary for this. In the



case of a subcutaneous or buccal administration, substance quantities of 5 - 500 nmol are necessary depending on the galenic form and intended duration of action.

The exendin analogues according to the invention or pharmacologically acceptable salts thereof are preferably stored as sterile lyophilisates and mixed with a suitable isotonic solution before administration. The analogues can then be injected, infused or optionally also absorbed through the mucous membranes in this form. The conventional isotonic aqueous systems that are suitable for injection or infusion which contain common additives for injection solutions such as stabilizers and solubilizers can be used as solvents. Physiological saline solution or optionally solutions made isotonic by buffers are preferred in this case.

Additives are, for example, tartrate or citrate buffer, ethanol, complexing agents (such as ethylene diamintetraacetic acid and non-toxic salts thereof), high molecular polymers (such as liquid polyethylene oxide) to regulate the viscosity. Liquid carrier substances for injection solutions must be sterile and are preferably filled into ampoules. Solid carrier substances are for example starch, lactose, mannitol, methyl cellulose, talcum, highly dispersed silicic acids, higher molecular fatty acids (such as stearic acid), gelatin, agar-agar, calcium phosphate, magnesium stearate, animal or vegetable fats, solid high molecular polymers (such as polyethylene glycol); suitable preparations for oral administration can if desired contain flavourings or sweeteners. For nasal administration surfactants can be added to improve absorption through the nasal mucous membrane e.g. cholic acid, taurocholic acid, chenodeoxycholic acid, glycolic



acid, dehydrocholic acid, deoxycholic acid and cyclodextrins.

The daily dose to be administered is in a range of 150-500 nmol. The determination of the biological activity is based on measurements compared to international reference preparations for glucagon-like peptide-1, exendin-3 or exendin-4 in a conventional test procedure for glucagon-like peptide-1.

The exendin analogues according to the invention can be prepared by conventional processes in peptide synthesis as described for example in J.M. Steward and J.D. Young "Solid Phase Peptide Synthesis", 2nd ed., Pierce Chemical Co., Rockford, Illinois (1984) and in J. Meienhofer Hormonal Proteins and Peptides, Vol.2 Academic Press, New York (1973) for solid phase synthesis and in E. Schroder and K. Lubke "The Peptides", Vol.1, Academic Press, New York (1965) for liquid phase synthesis.

#### **General processes for peptide synthesis**

In general protected amino acids are added to a growing peptide chain for the synthesis of peptides. Either the amino group or the carboxyl group as well as any reactive group in the side chain of the first amino acid are protected. This protected amino acid is either coupled to an inert support or it can also be used in solution. The next amino acid in the peptide sequence is appropriately protected under conditions which favour the formation of an amide bond and is added to the first. After all desired amino acids have been coupled in the correct sequence, the protective groups and optionally the support phase are cleaved. The crude polypeptide that is



obtained is reprecipitated and preferably purified chromatographically to form the final product.

A preferred method for synthesizing analogues of physiologically active polypeptides with fewer than fourty amino acids comprises a solid phase peptide synthesis. In this method the  $\alpha$ -amino functions ( $N^\alpha$ ) and any reactive side chains are protected with acid-labile or base-labile groups. The protective groups that are used should be stable under the conditions for linking amide bonds but it should be possible to readily cleave them without impairing the polypeptide chain that has formed. Suitable protective groups for the  $\alpha$ -amino function include the following groups but are not limited to these: t-butyloxycarbonyl (Boc), benzyloxycarbonyl (Z), o-chlorbenzyloxycarbonyl, bi-phenylisopropylloxycarbonyl, tert.-amyloxycarbonyl (Amoc),  $\alpha,\alpha$ -dimethyl-3,5-dimethoxy-benzyloxycarbonyl, o-nitrosulfenyl, 2-cyano-t-butoxy-carbonyl, 9-fluorenylmethoxycarbonyl (Fmoc), 1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl (Dde) and the like. 9-Fluorenylmethoxycarbonyl (Fmoc) is preferably used as the  $N^\alpha$ -protective group.

Suitable side chain protective groups include the following but are not limited to these: acetyl, allyl (All), allyloxycarbonyl (Alloc), benzyl (Bzl), benzyloxycarbonyl (Z), t-butyloxycarbonyl (Boc), benzyloxymethyl (Bom), o-bromobenzyloxycarbonyl, t-butyl (tBu), t-butyldimethylsilyl, 2-chlorobenzyl, 2-chlorobenzyloxycarbonyl (2-CIZ), 2,6-dichlorobenzyl, cyclohexyl, cyclopentyl, 1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)-ethyl (Dde), isopropyl, 4-methoxy-2,3,6-trimethylbenzylsulfonyl (Mtr), 2,3,5,7,8-pentamethylchroman-6-sulfonyl (Pmc), pivalyl, tetrahydropyran-2-yl, tosyl (Tos),



2,4,6-trimethoxybenzyl, trimethylsilyl and trityl (Trt).

In the solid phase synthesis the C-terminal amino acid is coupled as the first to a suitable support material. Suitable support materials are those which are inert towards the reagents and reaction conditions for the stepwise condensation and cleavage reactions and which do not dissolve in the reaction media that are used. Examples of commercially available support materials include styrene/divinylbenzene copolymers which have been modified with reactive groups and/or polyethylene glycol and also chloromethylated styrene/divinylbenzene copolymers, hydroxymethylated or aminomethylated styrene/divinylbenzene copolymers and the like. Polystyrene (1 %)-divinylbenzene or TentaGel® (Rapp Polymere, Tübingen) derivatized with 4-benzyloxybenzyl-alcohol (Wang-anchor (Wang, S.S. 1973)) or 2-chlorotrityl chloride (Barlos, K. et al. 1989) is preferably used if it is intended to prepare the peptidic acid. In the case of the peptide amide, polystyrene (1 %) divinylbenzene or TentaGel® derivatized with 5-(4'-aminomethyl)-3',5'-dimethoxy-phenoxy)valeric acid (PAL-anchor) (Albericio, F. et al. 1987) or p-(2,4-dimethoxyphenyl-amino methyl)-phenoxy group (Rink-Amid anchor (Rink, H. 1987)) is preferred.

The linkage to the polymeric support can be achieved by reacting the C-terminal Fmoc-protected amino acid with the support material with the addition of an activation reagent in ethanol, acetonitrile, N,N-dimethylformamide (DMF), dichloromethane, tetrahydrofuran, N-methylpyrrolidone or similar solvents preferably in DMF at room temperature or elevated temperatures e.g. between 40°C and 60°C, preferably at room temperature and with reaction times of 2 to 72 hours, preferably about 2 x 2



hours.

The coupling of the  $N^\alpha$  protected amino acid preferably the Fmoc amino acid to the PAL, Wang or Rink anchor can for example be carried out with the aid of coupling reagents such as N,N'-dicyclohexylcarbodiimide (DCC), N,N'-diisopropylcarbodiimide (DIC) or other carbodiimides, 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) or other uronium salts, o-acyl-ureas, benzotriazol-1-yl-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBOP) or other phosphonium salts, N-hydroxysuccinimides, other N-hydroxyimides or oximes in the presence or also in the absence of 1-hydroxybenzotriazole or 1-hydroxy-7-azabenzotriazole preferably with the aid of TBTU with addition of HOBt, with or without the addition of a base such as for example diisopropylethylamine (DIEA), triethylamine or N-methylmorpholine, preferably diisopropylethylamine with reaction times of 2 to 72 hours, preferably 3 hours in a 1.5 to 3-fold excess of the amino acid and the coupling reagents, preferably in a 2-fold excess and at temperatures between about 10°C and 50°C, preferably 25°C in a solvent such as dimethylformamide, N-methylpyrrolidone or dichloromethane, preferably dimethylformamide. Instead of the coupling reagents it is also possible to use the active esters (e.g. pentafluorophenyl, p-nitrophenyl or the like), the symmetric anhydride of the  $N^\alpha$ -Fmoc-amino acid, its acid chloride or acid fluoride under the conditions described above.

The  $N^\alpha$ -protected amino acid, preferably the Fmoc amino acid is preferably coupled to the 2-chlorotrityl resin in dichloromethane with the addition of DIEA with reaction times of 10 to 120 minutes, preferably 20



minutes but is not limited to the use of this solvent and this base.

The successive coupling of the protected amino acids can be carried out according to conventional methods in peptide synthesis typically in an automated peptide synthesizer. After cleavage of the  $N^{\alpha}$ -Fmoc protective group of the coupled amino acid on the solid phase by treatment with piperidine (10 % to 50 %) in dimethylformamide for 5 to 20 minutes, preferably 2x2 minutes with 50 % piperidine in DMF and 1x15 minutes with 20 % piperidine in DMF, the next protected amino acid in a 3 to 10-fold excess, preferably in a 10-fold excess is coupled to the previous amino acid in an inert, non-aqueous, polar solvent such as dichloromethane, DMF or mixtures of the two, preferably DMF and at temperatures between about 10°C and 50°C, preferably at 25°C. The reagents that have already been mentioned for coupling the first  $N^{\alpha}$ -Fmoc amino acid to the PAL, Wang or Rink anchor are suitable as coupling reagents. Active esters of the protected amino acid, or chlorides or fluorides or symmetric anhydrides thereof can also be used as an alternative.

At the end of the solid phase synthesis the peptide is cleaved from the support material while simultaneously cleaving the side chain protecting groups. Cleavage can be carried out with trifluoroacetic acid or other strongly acidic media with addition of 5 % - 20 % v/v scavengers such as dimethylsulfide, ethylmethylsulfide, thioanisole, thiocresol, m-cresol, anisole ethanedithiol, phenol or water, preferably 15 % v/v dimethylsulfide/ethanedithiol/m-cresol 1:1:1 within 0.5 to 3 hours, preferably 2 hours. Peptides with fully protected side chains are obtained by cleaving the 2-chlorotrityl



anchor with glacial acetic acid/trifluoroethanol/dichloromethane 2:2:6. The protected peptide can be purified by chromatography on silica gel. If the peptide is linked to the solid phase via the Wang anchor and if it is intended to obtain a peptide with a C-terminal alkylamidation, the cleavage can be carried out by aminolysis with an alkylamine or fluoroalkylamine. The aminolysis is carried out at temperatures between about -10°C and 50°C, preferably about 25°C and reaction times between about 12 and 24 hours, preferably about 18 hours. In addition the peptide can also be cleaved from the support by re-esterification e.g. with methanol.

The acidic solution that is obtained is admixed with a 3 to 20-fold amount of cold ether or n-hexane, preferably a 10-fold excess of diethyl ether, in order to precipitate the peptide and hence to separate the scavengers and cleaved protective groups that remain in the ether. A further purification can be carried out by re-precipitating the peptide several times from glacial acetic acid. The precipitate that is obtained is taken up in water or tert. butanol or mixtures of the two solvents, preferably a 1:1 mixture of tert.-butanol/water and freeze-dried.

The peptide obtained can be purified by some or all of the following chromatographic methods: ion exchange over a weakly basic resin in the acetate form; hydrophobic adsorption chromatography on non-derivatized polystyrene/divinylbenzene copolymers (e.g. Amberlite® XAD); adsorption chromatography on silica gel; ion exchange chromatography e.g. on carboxymethyl cellulose; distribution chromatography e.g. on Sephadex® G-25; countercurrent distribution chromatography; or high pressure liquid chromatography (HPLC) in particular





reversed-phase HPLC on octyl or octadecylsilylsilica (ODS) phases.

In summary part of the present invention encompasses processes for the preparation of polypeptides and pharmaceutically usable salts thereof. These processes which lead to physiologically active shortened homologues and analogues of exendin-3 or exendin-4 with the above-mentioned preferred chain lengths and modifications comprise processes for the sequential condensation of protected amino acids on a suitable support material, methods for cleaving the support and protective groups and for purifying the crude peptides that are obtained.

The amino acid analysis was carried out with an amino acid analyzer 420 A from the Applied Biosystems Company (Weiterstadt). 50 to 1000 pmol of the sample to be analysed was applied to the sample carrier in 10 to 40  $\mu$ l solution and subsequently fully automatically hydrolysed for 90 minutes in the gas phase at 160°C with 6 N hydrochloric acid, derivatized with phenylisothiocyanate and analysed on-line by a microbore HPLC. Mass spectroscopic examinations were carried out on an API III triple-quadrupole mass spectrometer (SCIEX; Thornhill, Canada) equipped with an ion spray ion source.

The protected amino acid derivatives can for example be obtained from Novabiochem GmbH (Bad Soden).

The following examples only represent an illustrative selection of the inventive thought and not a limitation of the subject matter of the invention.



**Exempl 1**

HGEGTFTSDLSKQ-Nle-EEEAVRLFIEWLKNGR-NH<sub>2</sub> (SEQ ID NO.3)  
[Nle<sup>14</sup>, Arg<sup>30</sup>]-exendin-4-(1-30)-NH<sub>2</sub>

Example 1 was synthesized in a 0.02 mmol batch by the solid phase method on 5-(4'-aminomethyl)-3',5'-dimethoxyphenoxy)valerianyl-alanyl-aminomethyl-polystyrene(1 %)divinylbenzene (loading: 0.5 mmol/g) on a multiple automated peptide synthesizer SyRo II from the MultiSyn Tech Company (Bochum). The  $\alpha$ -amino functional groups of the amino acids were protected with 9-fluorenylmethoxycarbonyl (Fmoc). The side chain protective groups were t-butyl (tBu) for Asp, Glu, Ser and Thr, trityl (Trt) for Asn, Gln and His, t-butyloxy-carbonyl (Boc) for Lys and Trp and 2,2,5,7,8-penta-methylchroman-6-sulfonyl (Pmc) for Arg. The protected amino acids were coupled sequentially in a 10-fold excess using double couplings of 2 times 40 minutes duration and using N,N-diisopropylcarbodiimide/1-hydroxybenzo-triazole as activation reagents. The peptide was cleaved from the polymeric support while simultaneously cleaving the protective groups in trifluoroacetic acid (85 %) in the presence of 15 % ethanedithiol/dimethylsulfide/m-cresol (1:1:1 v/v/v) for 120 minutes at room temperature. Subsequently the peptide was precipitated with anhydrous diethyl ether and then washed several times with anhydrous diethyl ether to completely remove the thiols. Freeze-drying of the precipitate from water/tert. butanol (1:1) yielded 62 mg of the crude peptide. The crude peptide was purified within 30 minutes by reversed-phase HPLC with a gradient of 37 % to 42 % acetonitrile/0.9 % TFA. The eluate was evaporated, lyophilized and gave a yield of 29 mg of a white solid with a purity of  $\geq 97$  %.



Amino acid analysis: Ala 1,08 (1); Asx 1,91 (2); Glx 6,10 (6); Phe 1,78 (2); Gly 3,10 (3); His 1,00 (1); Ile 0,88 (1); Lys 2,02 (2); Leu 3,24 (3); Nle 1,10 (1); Arg 1,98 (2); Ser 2,04 (2); Thr 1,99 (2); Val 0,91 (1); Trp 0,87 (1)

ESI-MS: 3488.2

## Example 2

HGEGTFTSDLSKQ-Nle-EEEAVRLFIEWLKNGY-NH<sub>2</sub> (SEQ ID No. 4)  
[Nle<sup>14</sup>, Tyr<sup>30</sup>]-exendin-4-(1-30)-NH<sub>2</sub>

Example 2 was synthesized in a 0.0076 mmol batch by the solid phase method on TentaGel<sup>®</sup> (Rapp Polymers, Tübingen) which was derivatized with a Rink-amide anchor (4-(2',4'-dimethoxyphenyl-aminomethyl)-phenoxy group) (loading: 0.18 mmol/g) on a multiple automated peptide synthesizer SyRo II from the MultiSynTech Company (Bochum). The protected amino acids that were used were analogous to example 1. The protected amino acids were sequentially coupled in an eight-fold excess with single couplings of 40 minutes duration at 40°C and while stirring. 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU)/1-hydroxybenzotriazole were used as activation reagents with the addition of diisopropylethylamine. The peptide was cleaved and purified analogously to example 1. 18.1 mg of a white solid with a purity of > 95 % was obtained.

Amino acid analysis: Ala 1,03 (1); Asx 1,90 (2); Glx 6,24 (6); Phe 1,94 (2); Gly 3,12 (3); His 1,02 (1); Ile 1,09 (1); Lys 2,01 (2); Leu 3,06 (3); Nle 1,08 (1); Arg 0,97 (1); Ser 1,98 (2); Thr 1,80 (2); Val 0,93 (1); Trp 1,01 (1); Tyr 0,90 (1).



ESI-MS: 3494.8

**Example 3**

HSDGTFTSDLSKQ-Nle-EEEEAVRLFIEWLKNGR-NH<sub>2</sub> (SEQ ID No. 5)  
[Nle<sup>14</sup>, Arg<sup>30</sup>]-exendin-3-(1-30)-NH<sub>2</sub>

Example 3 was synthesized analogously to the method described for example 2. 17.6 mg of a white solid with a purity of  $\geq 99$  % was obtained.

Amino acid analysis: Ala 0,99 (1); Asx 2,98 (3); Glx 5,16 (5); Phe 2,08 (2); Gly 2,16 (2); His 0,95 (1); Ile 1,03 (1); Lys 2,04 (2); Leu 2,91 (3); Nle 1,05 (1); Arg 1,04 (1); Ser 3,00 (3); Thr 2,05 (2); Val 1,01 (1); Trp 1,18 (1); Tyr 0,98 (1).

ESI-MS: 3504,4

**Example 4**

HGEGTFTSDLSKQMEEEEAVRLFIEWLKNGR-NH<sub>2</sub> (SEQ ID No. 6)  
[Arg<sup>30</sup>]-exendin-4-(1-30)-NH<sub>2</sub>

Example 4 was synthesized analogously to the method described for example 1. 17.9 mg of a white solid with a purity of  $\geq 96$  % was obtained.

Amino acid analysis: Ala 0,96 (1); Asx 2,01 (2); Glx 6,00 (6); Phe 1,80 (2); Gly 3,21 (3); His 0,96 (1); Ile 1,07 (1); Lys 1,92 (2); Leu 2,98 (3); Met 1,06 (1); Arg 1,90 (2); Ser 1,91 (2); Thr 2,09 (2); Val 0,97 (1); Trp 0,84 (1).

ESI-MS: 3508,4



**Exempl 5**

GEGTFTSDLSKQ-Nle-EEEAVRLFIEWLKNGR-NH<sub>2</sub> (SEQ ID No. 7)  
[Nle<sup>14</sup>, Arg<sup>30</sup>]-exendin-4-(2-30)-NH<sub>2</sub>

Example 5 was synthesized analogously to the method described for example 2. 13.2 mg of a white solid with a purity of  $\geq 97$  % was obtained.

Amino acid analysis: Ala 1,04 (1); Asx 1,98 (2); Glx 6,08 (6); Phe 1,86 (2); Gly 2,91 (3); Ile 0,96 (1); Lys 1,84 (2); Leu 2,98 (3); Nle 1,04 (1); Arg 1,90 (2); Ser 1,94 (2); Thr 1,92 (2); Val 0,96 (1); Trp 0,85 (1).

ESI-MS: 3350,8

**Example 6**

HGEGTFTSDLSKQMEEEAVRAFIIEWLKNGR-NH<sub>2</sub> (SEQ ID No. 8)  
[Ala<sup>21</sup>, Arg<sup>30</sup>]-exendin-4-(1-30)-NH<sub>2</sub>

Example 6 was synthesized analogously to the method described for example 1. 11.1 mg of a white solid with a purity of  $\geq 95$  % was obtained.

Amino acid analysis: Ala 2,08 (2); Asx 1,93 (2); Glx 6,07 (6); Phe 1,74 (2); Gly 2,97 (3); His 0,98 (1); Ile 0,87 (1); Lys 2,15 (2); Leu 2,02 (2); Met 0,96 (1); Arg 2,13 (2); Ser 1,87 (2); Thr 2,07 (2); Val 1,04 (1); Trp 0,87 (1).

ESI-MS: 3466,3



**Example 7**

HGEGTFTSDLSKQMEEEEAVRLFIEWLKAGR-NH<sub>2</sub> (SEQ ID No. 9)  
[Ala<sup>28</sup>, Arg<sup>30</sup>]-exendin-4-(1-30)-NH<sub>2</sub>

Example 7 was synthesized analogously to the method described for example 1. 15.0 mg of a white solid with a purity of  $\geq 97$  % was obtained.

Amino acid analysis: Ala 1,98 (2); Asx 0,98 (1); Glx 6,22 (6); Phe 1,92 (2); Gly 3,03 (3); His 0,99 (1); Ile 1,03 (1); Lys 2,05 (2); Leu 3,03 (3); Met 0,96 (1); Arg 1,84 (2); Ser 1,98 (2); Thr 2,09 (2); Val 1,01 (1); Trp 0,72 (1).

ESI-MS: 3465,4

**Example 8**

HGEGTFTSDLSKQMEEEEAVRAFIEWLKAGR-NH<sub>2</sub> (SEQ ID No. 10)  
[Ala<sup>21,28</sup>, Arg<sup>30</sup>]-exendin-4-(1-30)-NH<sub>2</sub>

Example 8 was synthesized analogously to the method described for example 1. 18.4 mg of a white solid with a purity of  $\geq 95$  % was obtained.

Amino acid analysis: Ala 3,12 (3); Asx 0,99 (1); Glx 6,04 (6); Phe 1,80 (2); Gly 3,00 (3); His 0,96 (1); Ile 1,02 (1); Lys 1,84 (2); Leu 1,97 (2); Met 0,98 (1); Arg 2,03 (2); Ser 1,91 (2); Thr 1,88 (2); Val 0,99 (1); Trp 0,99 (1).

ESI-MS: 3423,3



### Example 9

It was possible to prepare the following exendin derivatives in high purity in an analogous manner.

(Ex-4 = exendin-4, Ex-3 = exendin-3)

Exendin-Derivative	Seq.	Sequenz
$[A^{14}, R^{30}]$ -Ex-4-(1-30)-OH	11	HGEGTFTSDLSKQAE $\epsilon$ EEAVRLFIEWLKNGR-OH
Ac-[Ile <sup>14</sup> , R <sup>30</sup> ]-Ex-4-(1-30)-NH <sub>2</sub>	12	Ac-HGEGTFTSDLSKQIle $\epsilon$ EEAVRLFIEWLKNGR-NH <sub>2</sub>
[Nle <sup>14</sup> ]-Ex-4-(1-27)-NH <sub>2</sub>	13	HGEGTFTSDLSKQNle $\epsilon$ EEAVRLFIEWLK-NH <sub>2</sub>
$[A^{14,29}, R^{30}]$ -Ex-3-(1-30)-NH <sub>2</sub>	14	HSDGTFTSDLSKQAE $\epsilon$ EEAVRLFIEWLKNGR-NH <sub>2</sub>
$[A^{14,27}, R^{30}]$ -Ex-3-(1-30)-NH <sub>2</sub>	15	HSDGTFTSDLSKQAE $\epsilon$ EEAVRLFIEWLANGR-NH <sub>2</sub>
$[A^{14,26}, R^{30}]$ -Ex-3-(1-30)-NH <sub>2</sub>	16	HSDGTFTSDLSKQAE $\epsilon$ EEAVRLFIEWAKNGR-NH <sub>2</sub>
$[A^2, Nle^{14}, R^{30}]$ -Ex-4-(1-30)-NH <sub>2</sub>	17	HAEGTFTSDLSKQNle $\epsilon$ EEAVRLFIEWLKNGR-NH <sub>2</sub>
$[C^2, Nle^{14}, R^{30}]$ -Ex-4-(1-30)-NH <sub>2</sub>	18	HCEGTFTSDLSKQNle $\epsilon$ EEAVRLFIEWLKNGR-NH <sub>2</sub>
$[D^2, Nle^{14}, R^{30}]$ -Ex-4-(1-30)-NH <sub>2</sub>	19	HDEGTFTSDLSKQNle $\epsilon$ EEAVRLFIEWLKNGR-NH <sub>2</sub>
$[E^2, Nle^{14}, R^{30}]$ -Ex-4-(1-30)-NH <sub>2</sub>	20	HEGTFTSDLSKQNle $\epsilon$ EEAVRLFIEWLKNGR-NH <sub>2</sub>
$[F^2, Nle^{14}, R^{30}]$ -Ex-4-(1-30)-NH <sub>2</sub>	21	HFEGTFTSDLSKQNle $\epsilon$ EEAVRLFIEWLKNGR-NH <sub>2</sub>
$[H^2, Nle^{14}, R^{30}]$ -Ex-4-(1-30)-NH <sub>2</sub>	22	HHEGTFTSDLSKQNle $\epsilon$ EEAVRLFIEWLKNGR-NH <sub>2</sub>
$[I^2, Nle^{14}, R^{30}]$ -Ex-4-(1-30)-NH <sub>2</sub>	23	HIETFTSDLSKQNle $\epsilon$ EEAVRLFIEWLKNGR-NH <sub>2</sub>



$[K^2, Nle^{14}, R^{30}]$ -Ex-4-(1-30)-NH <sub>2</sub>	24	HKEGTFTSDLSKQN1eEEEAVRLFIEWLKN GR-NH <sub>2</sub>
$[L^2, Nle^{14}, R^{30}]$ -Ex-4-(1-30)-NH <sub>2</sub>	25	HLEGTFTSDLSKQN1 EEEAVRLFIEWLKN GR-NH <sub>2</sub>
$[M^2, Nle^{14}, R^{30}]$ -Ex-4-(1-30)-NH <sub>2</sub>	26	HMEGTFTSDLSKQN1eEEEAVRLFIEWLKN GR-NH <sub>2</sub>
$[N^2, Nle^{14}, R^{30}]$ -Ex-4-(1-30)-NH <sub>2</sub>	27	HNEGTFTSDLSKQN1eEEEAVRLFIEWLKN GR-NH <sub>2</sub>
$[P^2, Nle^{14}, R^{30}]$ -Ex-4-(1-30)-NH <sub>2</sub>	28	HPEGTFTSDLSKQN1eEEEAVRLFIEWLKN GR-NH <sub>2</sub>
$[Q^2, Nle^{14}, R^{30}]$ -Ex-4-(1-30)-NH <sub>2</sub>	29	HQEGTFTSDLSKQN1eEEEAVRLFIEWLKN GR-NH <sub>2</sub>
$[R^2, Nle^{14}, R^{30}]$ -Ex-4-(1-30)-NH <sub>2</sub>	30	HREGTFTSDLSKQN1eEEEAVRLFIEWLKN GR-NH <sub>2</sub>
$[S^2, Nle^{14}, R^{30}]$ -Ex-4-(1-30)-NH <sub>2</sub>	31	HSEGTFTSDLSKQN1eEEEAVRLFIEWLKN GR-NH <sub>2</sub>
$[T^2, Nle^{14}, R^{30}]$ -Ex-4-(1-30)-NH <sub>2</sub>	32	HTEGTFTSDLSKQN1eEEEAVRLFIEWLKN GR-NH <sub>2</sub>
$[V^2, Nle^{14}, R^{30}]$ -Ex-4-(1-30)-NH <sub>2</sub>	33	HVEGTFTSDLSKQN1eEEEAVRLFIEWLKN GR-NH <sub>2</sub>
$[W^2, Nle^{14}, R^{30}]$ -Ex-4-(1-30)-NH <sub>2</sub>	34	HWEGTFTSDLSKQN1eEEEAVRLFIEWLKN GR-NH <sub>2</sub>
$[Y^2, Nle^{14}, R^{30}]$ -Ex-4-(1-30)-NH <sub>2</sub>	35	HYEGTFTSDLSKQN1eEEEAVRLFIEWLKN GR-NH <sub>2</sub>
$[A_{12}^{2,24}, G^{16}, E^{21}, K^{20,28}, Q^{17}, R^{30}, S^{27}, Y^{13}]$ -Ex-3-(1-30)-NH <sub>2</sub>	36	HADGTFTSDLSYMEGQAVKEFIAWLKGR -NH <sub>2</sub>
$[A^{14,25}, R^{30}]$ -Ex-3-(1-30)-NH <sub>2</sub>	37	HSDGTFTSDLSKQAEAAVRLFIEALKNGR -NH <sub>2</sub>
$[E^3, A^{14}, R^{30}]$ -Ex-3-(1-30)-NH <sub>2</sub>	38	HSEGTFTSDLSKQAEAAVRLFIEWLKNGR -NH <sub>2</sub>
$[A^1, V^{14}, R^{30}]$ -Ex-3-(1-30)-NH <sub>2</sub>	39	ASDGTFTSDLSKQVEAAVRLFIEWLKNGR -NH <sub>2</sub>
$[A^{3,14}, R^{30}]$ -Ex-4-(1-30)-NH <sub>2</sub>	40	HGAGTFTSDLSKQAEAAVRLFIEWLKNGR -NH <sub>2</sub>





$[A^{5,14}, R^{30}]$ -Ex-4-(1-30)-NH <sub>2</sub>	41	HGEGFTSDLSKQAE EEA VRL FIEWLKNGR -NH <sub>2</sub>
$[A^{14}, R^{30}, Y^5]$ -Ex-4-(1-30)-NH <sub>2</sub>	42	HGEGYFTSDLSKQAE EEA VRL FIEWLKNGR -NH <sub>2</sub>
$[A^{14}, R^{30}, Y^6]$ -Ex-4-(1-30)-NH <sub>2</sub>	43	HGEGTYTSDLSKQAE EEA VRL FIEWLKNGR -NH <sub>2</sub>
$[A^{14}, I^6, R^{30}]$ -Ex-4-(1-30)-NH <sub>2</sub>	44	HGEGTITSDLSKQAE EEA VRL FIEWLKNGR -NH <sub>2</sub>
$[A^{14}, R^{30}, S^7]$ -Ex-4-(1-30)-NH <sub>2</sub>	45	HGEGTFSSDLSKQAE EEA VRL FIEWLKNGR -NH <sub>2</sub>
$[A^{14}, R^{30}, Y^7]$ -Ex-4-(1-30)-NH <sub>2</sub>	46	HGEGTFYSDLSKQAE EEA VRL FIEWLKNGR -NH <sub>2</sub>
$[A^{14}, R^{30}, T^8]$ -Ex-4-(1-30)-NH <sub>2</sub>	47	HGEGTFTDLSKQAE EEA VRL FIEWLKNGR -NH <sub>2</sub>
$[A^{14}, R^{30}, Y^8]$ -Ex-4-(1-30)-NH <sub>2</sub>	48	HGEGTFTYDLSKQAE EEA VRL FIEWLKNGR -NH <sub>2</sub>
$[A^{14}, E^9, R^{30}]$ -Ex-4-(1-30)-NH <sub>2</sub>	49	HGEGTFTSELSKQAE EEA VRL FIEWLKNGR -NH <sub>2</sub>
$[A^{10,14}, R^{30}]$ -Ex-4-(1-30)-NH <sub>2</sub>	50	HGEGTFTSDASKQAE EEA VRL FIEWLKNGR -NH <sub>2</sub>
$[A^{11,14}, R^{30}]$ -Ex-4-(1-30)-NH <sub>2</sub>	51	HGEGTFTSDLAKQAE EEA VRL FIEWLKNGR -NH <sub>2</sub>
$[A^{12,14}, R^{30}]$ -Ex-4-(1-30)-NH <sub>2</sub>	52	HGEGTFTSDLSAQAE EEA VRL FIEWLKNGR -NH <sub>2</sub>
$[A^{13,14}, R^{30}]$ -Ex-4-(1-30)-NH <sub>2</sub>	53	HGEGTFTSDLSKAAE EEA VRL FIEWLKNGR -NH <sub>2</sub>
$[A^{14,15}, R^{30}]$ -Ex-4-(1-30)-NH <sub>2</sub>	54	HGEGTFTSDLSKQAE EEA VRL FIEWLKNGR -NH <sub>2</sub>
$[A^{14,16}, G^1, R^{30}, S^5]$ -Ex-3-(1-30)-NH <sub>2</sub>	55	GSDGSFTSDLSKQAE EEA VRL FIEWLKNGR -NH <sub>2</sub>
$[A^{14,17}, K^1, R^{30}]$ -Ex-4-(1-30)-NH <sub>2</sub>	56	KGEGTFTSDLSKQAE EEA VRL FIEWLKNGR -NH <sub>2</sub>
$[A^{14}, L^{18}, R^{30}]$ -Ex-4-(1-30)-NH <sub>2</sub>	57	HGEGTFTSDLSKQAE EEL VRL FIEWLKNGR -NH <sub>2</sub>



$[A^{14}, I^{19}, R^{30}]$ -Ex-4-(1-30)-NH <sub>2</sub>	58	HGEGTFTSDLSKQAEAAAIRLFIEWLKNGR-NH <sub>2</sub>
$[A^{14,20}, R^{30}]$ -Ex-4-(1-30)-NH <sub>2</sub>	59	HGEGTFTSDLSKQAEAAAVALFIEWLKNGR-NH <sub>2</sub>
$[A^{14}, R^{30}, Y^{22}]$ -Ex-3-(1-30)-NH <sub>2</sub>	60	HSDGTFTSDLSKQAEAAAVALRYIEWLKNGR-NH <sub>2</sub>
$[A^{14}, R^{30}, V^{23}]$ -Ex-4-(1-30)-NH <sub>2</sub>	61	HGEGTFTSDLSKQAEAAAVALFVFWLKNGR-NH <sub>2</sub>
$[A^{14}, L^{24}, R^{30}]$ -Ex-4-(1-30)-NH <sub>2</sub>	62	HGEGTFTSDLSKQAEAAAVALFILWLKNGR-NH <sub>2</sub>
$[A^{14,25}, R^{30}]$ -Ex-4-(1-30)-NH <sub>2</sub>	63	HGEGTFTSDLSKQAEAAAVALFIEALKNGR-NH <sub>2</sub>
$[A^{14,26}, R^{30}]$ -Ex-4-(1-30)-NH <sub>2</sub>	64	HGEGTFTSDLSKQAEAAAVALFIEWAKNGR-NH <sub>2</sub>
$[A^{14,27}, R^{30}]$ -Ex-4-(1-30)-NH <sub>2</sub>	65	HGEGTFTSDLSKQAEAAAVALFIEWLANGR-NH <sub>2</sub>
$[A^{14,29}, R^{30}]$ -Ex-4-(1-30)-NH <sub>2</sub>	66	HGEGTFTSDLSKQAEAAAVALFIEWLKNAR-NH <sub>2</sub>
$[A^{14}, R^{30}]$ -Ex-4-(1-30)-NH <sub>2</sub>	67	HGEGTFTSDLSKQAEAAAVALFIEWLKNGR-NH <sub>2</sub>
$[R^{30}]$ -Ex-3-(1-30)-NH <sub>2</sub>	68	HSDGTFTSDLSKQMEAAAVALFIEWLKNGR-NH <sub>2</sub>
$[Nle^{14}, Y^{30}]$ -Ex-3-(1-30)-NH <sub>2</sub>	69	HSDGTFTSDLSKQNleAAAVALFIEWLKNGY-NH <sub>2</sub>
$[Nle^{14}, R^{30}]$ -Ex-3-(1-30)-OH	70	HSDGTFTSDLSKQNleAAAVALFIEWLKNGR-OH
$[A^{14}, R^{30}]$ -Ex-3-(1-30)-NH <sub>2</sub>	71	HSDGTFTSDLSKQAEAAAVALFIEWLKNGR-NH <sub>2</sub>
$[Nle^{14}, R^{30}]$ -Ex-3-(2-30)-NH <sub>2</sub>	72	SDGTFTSDLSKQNleAAAVALFIEWLKNGR-NH <sub>2</sub>
$[Nle^{14}, R^{30}]$ -Ex-3-(3-30)-NH <sub>2</sub>	73	DGTFTSDLSKQNleAAAVALFIEWLKNGR-NH <sub>2</sub>
Ac- $[Nle^{14}, R^{30}]$ -Ex-3-(2-30)-NH <sub>2</sub>	74	Ac-SDGTFTSDLSKQNleAAAVALFIEWLKNGR-NH <sub>2</sub>



Ac-[Nle <sup>14</sup> , R <sup>30</sup> ]-Ex-3-(3-30)-NH <sub>2</sub>	75	AC-DGTFTSDLSKQNIeEEEEAVRLFIEWLKNGR-NH <sub>2</sub>
[Nle <sup>14</sup> ]-Ex-3-(1-27)-NH <sub>2</sub>	76	HSDGTFTSDLSKQNIeEEEEAVRLFIEWLK-NH <sub>2</sub>
[K <sup>2</sup> , P <sup>3</sup> , A <sup>14</sup> , R <sup>30</sup> ]-Ex-3-(1-30)-NH <sub>2</sub>	77	HKPGTFTSDLSKQNAEEEEAVRLFIEWLKNGR-NH <sub>2</sub>
[A <sup>2</sup> , Nle <sup>14</sup> , R <sup>30</sup> ]-Ex-3-(1-30)-NH <sub>2</sub>	78	HADGTFTSDLSKQNIeEEEEAVRLFIEWLKNGR-NH <sub>2</sub>
[C <sup>2</sup> , Nle <sup>14</sup> , R <sup>30</sup> ]-Ex-3-(1-30)-NH <sub>2</sub>	79	HCDGTFTSDLSKQNIeEEEEAVRLFIEWLKNGR-NH <sub>2</sub>
[D <sup>2</sup> , Nle <sup>14</sup> , R <sup>30</sup> ]-Ex-3-(1-30)-NH <sub>2</sub>	80	HDDGTFTSDLSKQNIeEEEEAVRLFIEWLKNGR-NH <sub>2</sub>
[E <sup>2</sup> , Nle <sup>14</sup> , R <sup>30</sup> ]-Ex-3-(1-30)-NH <sub>2</sub>	81	HEDGTFTSDLSKQNIeEEEEAVRLFIEWLKNGR-NH <sub>2</sub>
[F <sup>2</sup> , Nle <sup>14</sup> , R <sup>30</sup> ]-Ex-3-(1-30)-NH <sub>2</sub>	82	HFDGTFTSDLSKQNIeEEEEAVRLFIEWLKNGR-NH <sub>2</sub>
[G <sup>2</sup> , Nle <sup>14</sup> , R <sup>30</sup> ]-Ex-3-(1-30)-NH <sub>2</sub>	83	HGDGTFTSDLSKQNIeEEEEAVRLFIEWLKNGR-NH <sub>2</sub>
[H <sup>2</sup> , Nle <sup>14</sup> , R <sup>30</sup> ]-Ex-3-(1-30)-NH <sub>2</sub>	84	HHDGTFTSDLSKQNIeEEEEAVRLFIEWLKNGR-NH <sub>2</sub>
[I <sup>2</sup> , Nle <sup>14</sup> , R <sup>30</sup> ]-Ex-3-(1-30)-NH <sub>2</sub>	85	HIDGTFTSDLSKQNIeEEEEAVRLFIEWLKNGR-NH <sub>2</sub>
[K <sup>2</sup> , Nle <sup>14</sup> , R <sup>30</sup> ]-Ex-3-(1-30)-NH <sub>2</sub>	86	HKDGTFTSDLSKQNIeEEEEAVRLFIEWLKNGR-NH <sub>2</sub>
[L <sup>2</sup> , Nle <sup>14</sup> , R <sup>30</sup> ]-Ex-3-(1-30)-NH <sub>2</sub>	87	HLDGTFTSDLSKQNIeEEEEAVRLFIEWLKNGR-NH <sub>2</sub>
[M <sup>2</sup> , Nle <sup>14</sup> , R <sup>30</sup> ]-Ex-3-(1-30)-NH <sub>2</sub>	88	HMDGTFTSDLSKQNIeEEEEAVRLFIEWLKNGR-NH <sub>2</sub>
[N <sup>2</sup> , Nle <sup>14</sup> , R <sup>30</sup> ]-Ex-3-(1-30)-NH <sub>2</sub>	89	HNDGTFTSDLSKQNIeEEEEAVRLFIEWLKNGR-NH <sub>2</sub>
[P <sup>2</sup> , Nle <sup>14</sup> , R <sup>30</sup> ]-Ex-3-(1-30)-NH <sub>2</sub>	90	HPDGTFTSDLSKQNIeEEEEAVRLFIEWLKNGR-NH <sub>2</sub>
[Q <sup>2</sup> , Nle <sup>14</sup> , R <sup>30</sup> ]-Ex-3-(1-30)-NH <sub>2</sub>	91	HQDGTFTSDLSKQNI EEEEEAVRLFIEWLKNGR-NH <sub>2</sub>



$[R^2, Nle^{14}, R^{30}]$ -Ex-3-(1-30)-NH <sub>2</sub>	92	HRDGTFTSDLSKQNI $\epsilon$ EEEEAVRLFIEWLKN GR-NH <sub>2</sub>
$[T^2, Nle^{14}, R^{30}]$ -Ex-3-(1-30)-NH <sub>2</sub>	93	HTDGTFTSDLSKQNI $\epsilon$ EEEEAVRLFIEWLKN GR-NH <sub>2</sub>
$[V^2, Nle^{14}, R^{30}]$ -Ex-3-(1-30)-NH <sub>2</sub>	94	HVDGTFTSDLSKQNI $\epsilon$ EEEEAVRLFIEWLKN GR-NH <sub>2</sub>
$[W^2, Nle^{14}, R^{30}]$ -Ex-3-(1-30)-NH <sub>2</sub>	95	HWDGTFTSDLSKQNI $\epsilon$ EEEEAVRLFIEWLKN GR-NH <sub>2</sub>
$[Y^2, Nle^{14}, R^{30}]$ -Ex-3-(1-30)-NH <sub>2</sub>	96	HYDGTFTSDLSKQNI $\epsilon$ EEEEAVRLFIEWLKN GR-NH <sub>2</sub>
$[A^{3,14}, R^{30}]$ -Ex-3-(1-30)-NH <sub>2</sub>	97	HSAGTFTSDLSKQAE $\epsilon$ EEEEAVRLFIEWLKNGR -NH <sub>2</sub>
$[A^{5,14}, R^{30}]$ -Ex-3-(1-30)-NH <sub>2</sub>	98	HSDGAFTSDLSKQAE $\epsilon$ EEEEAVRLFIEWLKNGR -NH <sub>2</sub>
$[A^{14}, R^{30}, Y^5]$ -Ex-3-(1-30)-NH <sub>2</sub>	99	HSDGYFTSDLSKQAE $\epsilon$ EEEEAVRLFIEWLKNGR -NH <sub>2</sub>
$[A^{14}, R^{30}, Y^6]$ -Ex-3-(1-30)-NH <sub>2</sub>	100	HSDGTYTSDLSKQAE $\epsilon$ EEEEAVRLFIEWLKNGR -NH <sub>2</sub>
$[A^{14}, I^6, R^{30}]$ -Ex-3-(1-30)-NH <sub>2</sub>	101	HSDGTITSDLSKQAE $\epsilon$ EEEEAVRLFIEWLKNGR -NH <sub>2</sub>
$[A^{14}, R^{30}, S^7]$ -Ex-3-(1-30)-NH <sub>2</sub>	102	HSDGTFSSDLSKQAE $\epsilon$ EEEEAVRLFIEWLKNGR -NH <sub>2</sub>
$[A^{14}, R^{30}, Y^7]$ -Ex-3-(1-30)-NH <sub>2</sub>	103	HSDGTFYS $\epsilon$ SDLSKQAE $\epsilon$ EEEEAVRLFIEWLKNGR -NH <sub>2</sub>
$[A^{14}, R^{30}, T^8]$ -Ex-3-(1-30)-NH <sub>2</sub>	104	HSDGTFTTDL $\epsilon$ SKQAE $\epsilon$ EEEEAVRLFIEWLKNGR -NH <sub>2</sub>
$[A^{14}, R^{30}, Y^8]$ -Ex-3-(1-30)-NH <sub>2</sub>	105	HSDGTFTYDL $\epsilon$ SKQAE $\epsilon$ EEEEAVRLFIEWLKNGR -NH <sub>2</sub>
$[A^{14}, E^9, R^{30}]$ -Ex-3-(1-30)-NH <sub>2</sub>	106	HSDGTFTSEL $\epsilon$ SKQAE $\epsilon$ EEEEAVRLFIEWLKNGR -NH <sub>2</sub>
$[A^{10,14}, R^{30}]$ -Ex-3-(1-30)-NH <sub>2</sub>	107	HSDGTFTSDASKQAE $\epsilon$ EEEEAVRLFIEWLKNGR -NH <sub>2</sub>
$[A^{11,14}, R^{30}]$ -Ex-3(1-30)-NH <sub>2</sub>	108	HSDGTFTSDLAKQAE $\epsilon$ EEEEAVRLFIEWLKNGR -NH <sub>2</sub>



$[A^{12,14}, R^{30}]$ -Ex-3-(1-30)-NH <sub>2</sub>	109	HGEGTFTSDLSAQAE <sup>3</sup> EEAVRLFIEWLKNGR-NH <sub>2</sub>
$[A^{13,14}, R^{30}]$ -Ex-3-(1-30)-NH <sub>2</sub>	110	HSDGTFTSDLSKAA <sup>3</sup> EEAVRLFIEWLKNGR-NH <sub>2</sub>
$[A^{14,15}, R^{30}]$ -Ex-3-(1-30)-NH <sub>2</sub>	111	HSDGTFTSDLSKQA <sup>3</sup> EEAVRLFIEWLKNGR-NH <sub>2</sub>
$[A^{14,17}, K^1, R^{30}]$ -Ex-3-(1-30)-NH <sub>2</sub>	112	KSDGTFTSDLSKQA <sup>3</sup> EEAVRLFIEWLKNGR-NH <sub>2</sub>
$[A^{14}, L^{18}, R^{30}]$ -Ex-3-(1-30)-NH <sub>2</sub>	113	HSDGTFTSDLSKQA <sup>3</sup> EEELVRLFIEWLKNGR-NH <sub>2</sub>
$[A^{14}, I^{19}, R^{30}]$ -Ex-3-(1-30)-NH <sub>2</sub>	114	HSDGTFTSDLSKQA <sup>3</sup> EEEAIRLFIEWLKNGR-NH <sub>2</sub>
$[A^{14,20}, R^{30}]$ -Ex-3-(1-30)-NH <sub>2</sub>	115	HSDGTFTSDLSKQA <sup>3</sup> EEEAVALFIEWLKNGR-NH <sub>2</sub>
$[A^{14}, R^{30}, Y^{22}]$ -Ex-3-(1-30)-NH <sub>2</sub>	116	HSDGTFTSDLSKQA <sup>3</sup> EEEA <sup>3</sup> VR <sup>3</sup> LYIEWLKNGR-NH <sub>2</sub>
$[A^{14}, R^{30}, V^{23}]$ -Ex-3-(1-30)-NH <sub>2</sub>	117	HSDGTFTSDLSKQA <sup>3</sup> EEEA <sup>3</sup> VR <sup>3</sup> LFV <sup>3</sup> EWLKNGR-NH <sub>2</sub>
$[A^{14}, L^{24}, R^{30}]$ -Ex-3-(1-30)-NH <sub>2</sub>	118	HSDGTFTSDLSKQA <sup>3</sup> EEEA <sup>3</sup> VR <sup>3</sup> LFILWLKNGR-NH <sub>2</sub>
Suc-[Nle <sup>14</sup> , R <sup>30</sup> ]-Ex-3-(3-30)-NH <sub>2</sub>	119	Suc-DGTFTSDLSKQN <sup>1</sup> le <sup>3</sup> EEEA <sup>3</sup> VR <sup>3</sup> LFIEWLKNGR-NH <sub>2</sub>
[Nle <sup>14</sup> , R <sup>30</sup> ]-Ex-3-(1-30)-NH <sub>2</sub>	120	HSDGTFTSDLSKQN <sup>1</sup> le <sup>3</sup> EEEA <sup>3</sup> VR <sup>3</sup> LFIEWLKNGR-NH <sub>2</sub>



The following examples were synthesized in a 0.02 mmol batch by the solid phase method on RAM resin® (Rapp Polymere, Tübingen) in which aminomethylpolystyrene(1 %) divinylbenzene is derivatized with Rink amide anchor (4-(2',4'-dimethoxyphenyl-aminomethyl)-phenoxy group) (loading: 0.5 mmol/g). The syntheses were carried out on a multiple automated peptide synthesizer SyRo II from the MultiSynTech Company (Bochum). The protected amino acids that were used were analogous to those of example 1. The protected amino acids were sequentially coupled in a 5-fold excess with single couplings of 40 minutes duration at 40°C and while stirring. 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) with the addition of diisopropylethylamine was used as the activation reagent. The cleavage and purification of the peptide was carried out analogously to example 1. The yields, purities and analytical data of the peptides synthesized as described above are listed in the following two tables.

Table 1

Seq. ID	Yield [mg]	Purity [%]	ESI-MS
36	6,0	99	3343,3
38	12,8	99	3477,4
68	14,2	99	3523,4
69	14,1	> 99	3512,1
70	18,0	95	3506,0
71	22,4	95	3463,6
72	6,6	> 99	3368,0
73	17,8	> 99	3281,1
75	12,0	99	3323,1
76	14,0	99	3178,1
77	7,2	99	3486,6
78	23,8	95	3488,1



79	19,0	95	3520,1
80	15,2	95	3531,9
81	8,6	> 99	3545,9
82	23,6	98	3563,9
83	25,4	95	3475,6
84	10,4	> 99	3554,1
85	8,2	> 99	3530,1
86	10,4	95	3545,3
87	9,2	> 99	3530,1
88	13,6	> 99	3548,1
89	10,6	> 99	3531,0
90	9,4	96	3514,9
91	6,0	> 99	3545,4
92	15,4	> 99	3574,9
93	10,1	> 99	3519,7
94	9,4	> 99	3517,7
95	12,0	> 99	3604,7
96	9,8	95	3581,8
120	8,5	95	3505,6





Table 2

Amino acid analyses

Seq. ID	Ala	Arg	Asx	Glx	Gly	His	Ile	Leu	Lys	Nle	Phe	Ser	Thr	Trp	Val
36 <sup>a</sup>	3,18 (3)	0,99 (1)	1,95 (2)	3,04 (3)	2,94 (3)	0,89 (1)	1,03 (1)	2,14 (2)	2,09 (2)		1,95 (2)	3,06 (3)	1,88 (2)	0,99 (1)	1,94 (2)
38	2,08 (2)	2,10 (2)	2,00 (2)	6,06 (6)	2,04 (2)	0,81 (1)	1,01 (1)	3,25 (3)	2,13 (2)		1,96 (2)	3,08 (3)	1,82 (2)	0,94 (1)	1,02 (1)
68 <sup>b</sup>	1,02 (1)	2,14 (2)	3,16 (3)	5,06 (5)	1,71 (2)	0,93 (1)	1,02 (1)	3,21 (3)	2,08 (2)		1,93 (2)	3,00 (3)	1,88 (2)	0,98 (1)	1,02 (1)
69 <sup>c</sup>	1,02 (1)	1,12 (1)	2,99 (3)	4,94 (5)	1,81 (2)	0,77 (1)	1,12 (1)	3,26 (3)	2,12 (2)	1,04 (1)	1,99 (2)	2,91 (3)	1,81 (2)	1,06 (1)	1,03 (1)
70	1,08 (1)	2,15 (2)	2,96 (3)	5,07 (5)	1,84 (2)	0,85 (1)	1,06 (1)	3,23 (3)	2,06 (2)	0,92 (1)	1,99 (2)	2,01 (3)	1,84 (2)	1,05 (1)	1,04 (1)
71	1,98 (2)	2,06 (2)	3,14 (3)	4,98 (5)	1,95 (2)	0,85 (1)	1,01 (1)	3,20 (3)	2,08 (2)		1,94 (2)	3,09 (3)	1,90 (2)	1,03 (1)	0,96 (1)
72	1,02 (1)	2,12 (2)	3,06 (3)	5,02 (5)	1,94 (2)		1,02 (1)	3,25 (3)	2,11 (2)	0,95 (1)	1,98 (2)	2,92 (3)	1,88 (2)	1,02 (1)	1,03 (1)
73	1,01 (1)	2,04 (2)	3,02 (3)	5,03 (5)	1,83 (2)		0,99 (1)	3,19 (3)	2,07 (2)	1,06 (1)	1,98 (2)	2,00 (2)	1,87 (2)	0,97 (1)	1,03 (1)





75	1,09 (1)	2,21 (2)	3,10 (3)	5,18 (5)	1,75 (2)	1,16 (1)	3,26 (3)	2,10 (2)	1,08 (1)	1,95 (2)	2,10 (2)	2,04 (2)	1,28 (1)	0,88 (1)
76	1,03 (1)	1,06 (1)	2,05 (2)	4,90 (5)	0,94 (1)	0,88 (1)	3,18 (3)	2,01 (2)	1,03 (1)	1,95 (2)	3,02 (3)	1,86 (2)	0,98 (1)	1,03 (1)
77 <sup>d</sup>	2,07 (2)	2,00 (2)	2,10 (2)	5,07 (5)	1,78 (2)	0,93 (1)	3,14 (3)	3,12 (3)		1,93 (2)	2,05 (2)	1,93 (2)	1,01 (1)	1,01 (1)
78	2,11 (2)	2,17 (2)	3,14 (3)	5,01 (5)	1,92 (2)	0,90 (1)	3,25 (3)	2,06 (2)	1,05 (1)	1,99 (2)	2,06 (2)	1,90 (2)	1,00 (1)	1,05 (1)
79 <sup>e</sup>	1,16 (1)	2,10 (2)	3,07 (3)	5,07 (5)	1,78 (2)	0,66 (1)	3,30 (3)	2,06 (2)	0,96 (1)	1,93 (2)	2,02 (2)	1,87 (2)	1,06 (1)	1,05 (1)
80	1,07 (1)	2,13 (2)	3,78 (4)	5,03 (5)	1,82 (2)	0,84 (1)	3,20 (3)	2,04 (2)	0,92 (1)	1,97 (2)	1,99 (2)	1,82 (2)	1,05 (1)	1,03 (1)
81	1,04 (1)	2,06 (2)	3,08 (3)	5,96 (6)	2,01 (2)	0,82 (1)	3,17 (3)	2,08 (2)	0,95 (1)	2,04 (2)	2,05 (2)	1,89 (2)	1,01 (1)	0,96 (1)
82	1,09 (1)	1,96 (2)	3,01 (3)	4,99 (5)	1,89 (2)	1,02 (1)	3,14 (3)	2,04 (2)	0,91 (1)	2,74 (3)	1,98 (2)	1,85 (2)	0,98 (1)	0,99 (1)
83	1,08 (1)	2,22 (2)	3,09 (3)	5,01 (5)	2,85 (3)	0,83 (1)	3,23 (3)	2,13 (2)	1,12 (1)	1,99 (2)	2,09 (2)	1,86 (2)	0,99 (1)	1,03 (1)
84	1,01 (1)	1,98 (2)	3,09 (3)	5,01 (5)	1,71 (2)	1,69 (2)	3,18 (3)	2,07 (2)	0,94 (1)	1,99 (2)	1,90 (2)	1,84 (2)	0,98 (1)	1,01 (1)
85	1,07 (1)	2,12 (2)	3,11 (3)	5,03 (5)	1,88 (2)	0,93 (1)	3,23 (3)	2,11 (2)	1,11 (1)	1,97 (2)	2,03 (2)	1,94 (2)	0,97 (1)	1,02 (1)



86	1,03 (1)	2,11 (2)	3,07 (3)	5,03 (5)	2,10 (2)	0,88 (1)	1,11 (1)	3,21 (3)	3,00 (3)	0,98 (1)	1,97 (2)	2,05 (2)	1,88 (2)	1,01 (1)	0,99 (1)
87	1,11 (1)	2,16 (2)	3,13 (3)	5,03 (5)	1,94 (2)	0,89 (1)	1,09 (1)	4,05 (4)	2,23 (2)	0,93 (1)	1,97 (2)	2,07 (2)	1,92 (2)	0,95 (1)	1,00 (1)
88 <sup>f</sup>	1,03 (1)	2,14 (2)	3,16 (3)	5,07 (5)	1,71 (2)	0,93 (1)	1,02 (1)	3,21 (3)	2,09 (2)	0,94 (1)	1,93 (2)	2,01 (2)	1,88 (2)	0,99 (1)	1,02 (1)
89	1,08 (1)	1,97 (2)	3,89 (4)	5,06 (5)	1,95 (2)	0,88 (1)	1,03 (1)	3,19 (3)	2,08 (2)	0,92 (1)	1,94 (2)	2,03 (2)	1,87 (2)	0,99 (1)	0,97 (1)
90 <sup>g</sup>	1,00 (1)	2,09 (2)	3,18 (3)	5,04 (5)	1,97 (2)	0,86 (1)	1,02 (1)	3,23 (3)	2,10 (2)	1,01 (1)	1,96 (2)	2,09 (2)	1,92 (2)	1,04 (1)	0,97 (1)
91	1,04 (1)	2,08 (2)	3,07 (3)	5,73 (6)	1,87 (2)	0,80 (1)	1,05 (1)	3,21 (3)	2,10 (2)	1,05 (1)	1,93 (2)	1,95 (2)	1,79 (2)	1,11 (1)	1,06 (1)
92	1,01 (1)	3,11 (3)	3,02 (3)	5,03 (5)	1,83 (2)	0,82 (1)	0,99 (1)	3,19 (3)	2,07 (2)	1,06 (1)	1,97 (2)	2,00 (2)	1,87 (2)	0,97 (1)	1,03 (1)
93	1,05 (1)	2,12 (2)	3,02 (3)	5,04 (5)	1,98 (2)	0,82 (1)	1,02 (1)	3,28 (3)	2,14 (2)	1,00 (1)	1,96 (2)	2,06 (2)	2,75 (3)	0,95 (1)	1,03 (1)
94	1,07 (1)	2,17 (2)	3,04 (3)	5,08 (5)	1,72 (2)	0,82 (1)	1,14 (1)	3,20 (3)	2,06 (2)	1,06 (1)	1,92 (2)	2,06 (2)	2,00 (2)	1,26 (1)	1,72 (2)
95	1,05 (1)	2,18 (2)	3,04 (3)	5,04 (5)	1,84 (2)	0,80 (1)	1,04 (1)	3,31 (3)	2,14 (2)	0,95 (1)	1,96 (2)	2,02 (2)	1,88 (2)	1,76 (2)	1,14 (1)



	1,03	2,25	3,03	5,00	1,84	0,78	1,14	3,30	2,15	1,05	2,02	1,96	1,84	1,07	1,05
96 <sup>h</sup>	1,03 (1)	2,25 (2)	3,03 (3)	5,00 (5)	1,84 (2)	0,78 (1)	1,14 (1)	3,30 (3)	2,15 (2)	1,05 (1)	2,02 (2)	1,96 (2)	1,84 (2)	1,07 (1)	1,05 (1)
120	1,11 (1)	1,98 (2)	3,05 (3)	5,06 (5)	1,91 (2)	1,03 (1)	1,03 (1)	3,18 (3)	2,07 (2)	0,93 (1)	1,95 (2)	2,94 (3)	1,88 (2)	0,99 (1)	1,00 (1)
a	methionine 0,96 (1); tyrosine 0,86 (1)														
b	methionine 0,94 (1)														
c	tyrosine 0,82 (1)														
d	proline 1,02 (1)														
e	cysteine could not be detected under the given hydrolysis conditions														
f	methionine 0,93 (1)														
g	proline 0,86 (1)														
h	tyrosine 0,87 (1)														

### **Example 10: Pharmacological data**

#### **Peptide metabolism in ectopeptidase preparations or in kidney microvilli membrane preparations**

##### **Background**

A group of ectopeptidases is responsible for the post-secretory metabolism of peptide hormones. These enzymes are bound to the plasma membranes of various cell types. Their active site is oriented towards the extracellular space. In addition these enzymes are present in high concentrations in the brush border membranes of the proximal renal tubuli. Renal brush border microvilli membranes (BBM) are therefore a suitable source for the relevant ectopeptidases and can be used as an in vitro test for the metabolic stability of synthetic peptides. Alternatively it is possible to use ectopeptidase preparations. The human neutral endopeptidase 24.11 as well as dipeptidyl peptidase IV were used as examples since GLP-1 is a substrate of both these ectopeptidases.

##### **Preparation of brush border microvilli membranes**

Microvilli membranes of the rat and pig kidney cortex are isolated by means of subcellular fractionation using the differential centrifugation method (Booth and Kenny (1975)). 4 Brush border ectopeptidases are examined fluorimetrically and other marker enzymes are measured colorimetrically in order to assess the degree of purity and the yield of membranes.

##### **Ectopeptidase preparations**

Purified human neutral endopeptidase 24.11 was obtained in a recombinant form from Genentech (San Francisco, USA), dipeptidyl peptidase IV was obtained as an isolate



from human placenta from Calbiochem (Bad Soden).

#### Incubation protocol

Microvilli membranes (0.5 - 1  $\mu$ g protein) or the respective ectopeptidase preparation (60-300 ng) were incubated with 10  $\mu$ g peptide (about 3 nmol) in 100  $\mu$ l HEPES buffer (50 mM, pH 7.4) which contained 50 mM NaCl. The reactions were terminated at predetermined times (duration up to 1 hour) by boiling. Subsequently the samples were centrifuged (10,000 x g), diluted with 150  $\mu$ l 0.1 % TFA and analysed by means of reversed phase (RP) HPLC. Each sample was determined in duplicate.

#### HPLC analysis

A system with the following components was used for HPLC analysis: A 2248 low pressure pump (Pharmacia-LKB, Freiburg), a WISP 10B autoinjector (Millipore-Waters, Eschborn), a UV detector SP-4 (Gynkotec, Berlin), a low pressure mixing system (Pharmacia-LKB, Freiburg) and a program manager software control (Pharmacia-LKB, Freiburg). The separations were carried out over Lichrospher C-8, 5  $\mu$ , 4 x 124 mm (Merck, Darmstadt) with a binary gradient using the mobile solvents A: 0.1 % trifluoroacetic acid (TFA) and B: acetonitrile: water: TFA (70:29:0.1). After injection of 244  $\mu$ l of the sample solution onto a column equilibrated with the mobile solvent A, the incubation products were eluted with a linear gradient of 0 % to 80 % B in 80 min and detected at 215 nm UV absorption.

#### Calculation of the proteolysis rates

Two measurements were carried out for each incubation period of each peptide and the average peak height of the substrate peak was plotted versus time. Using GLP-1 as an example it was possible to show that the peak



height is linearly proportional to the quantity of the peptid in the sample solution. Furthermore a linear decrease of the peak height with time was observed within the first hour of incubation with the microvilli membranes or the peptidases. Hence the proteolysis rate is determined from the decrease in the height of the substrate peak and is expressed as [ $\mu$ mol substrate/mg protein/minute].

#### Degradation stability of exendin analogues

##### Incubation with human neutral endopeptidase 24.11b

[Nle<sup>14</sup>,Arg<sup>30</sup>]-exendin-4-(1-30)-NH<sub>2</sub> (SEQ ID No. 3) was incubated with the neutral endopeptidase 24.11 as described above and the degradation rate was determined. GLP-1-(7-36)-NH<sub>2</sub> served as a control. The results are shown in Table 3.

Table 3

	Degradation rate	
	[mM/100ng/ml NEP24.11/ min]	
GLP1-(7-36)-NH <sub>2</sub>	0,0586	
[Nle <sup>14</sup> ,Arg <sup>30</sup> ]-Ex-4-(1-30)-NH <sub>2</sub> Exmp. 1	0,0083	

##### Incubations with dipeptidyl peptidase IV

The peptides listed in Table 4 were incubated with dipeptidyl peptidase IV (DDP-IV) as described above. Each of the incubations was terminated at the time when the GLP-1-(7-36)-NH<sub>2</sub> exhibited 50 % hydrolysis. The substrate peak of each peptide was collected from the rphPLC run and examined by mass spectroscopy in order to



exclude truncated products.

Table 4

Analogue	Seq.ID	Substrate for DDP-IV
[Ala <sup>2</sup> ,Nle <sup>14</sup> ,Arg <sup>30</sup> ]-Ex-3-(1-30)-NH <sub>2</sub>	78	no proteolysis
GLP1-(7-36)-NH <sub>2</sub>		50% proteolysis

#### Incubations with brush border microvilli membranes

The proteolysis rates which were calculated after incubation with brush border microvilli membranes (BBM) by the protocol described above are shown in Table 5. GLP-1-(7-36)-NH<sub>2</sub> served as a control.

Table 5

Analogue	Seq.ID	Degradation rate [ng peptide/min/mg BBM]
GLP1-(7-36)-NH <sub>2</sub>		880,00
[Lys <sup>2</sup> ,Nle <sup>14</sup> ,Arg <sup>30</sup> ]-Ex-3-(1-30)-NH <sub>2</sub>	86	2,05

#### Insulin secretion by isolated islet cells

#### Organ removal

The abdomen of anaesthetised (0.3 - 0.5 ml nembutal/isotonic saline solution 1:4 i.p.) mice is opened by a median incision and two side incisions, the peritoneum is immobilized and cut open at the costal arch along the diaphragm. All organs are inflated and stained red by injection of a neutral red solution into the left



ventricle. The pancreas is carefully removed along the stomach and duodenum up to the mesenteries. Until digestion the pancreas is placed in an ice-cooled petri dish in Hank's balanced salt solution (HBBS) and a few drops of neutral red.

#### Islet preparation

Two pancreases are dabbed with cellulose, placed in a tube, 5 ml freshly prepared collagenase solution (collagenase (Cl. histolyticum) 0.74 U/mg, Serva, 2 mg/ml in HBBS/water 1:9, pH 7.4) is added and they are incubated for 18 minutes at 37°C while shaking. Subsequently a centrifugation is carried out at 1000 rpm for 1 minute. The supernatant is discarded. In a second digestion step 5 ml collagenase solution (1 mg/ml) is incubated for 4 minutes, shaken and undigested tissue is sedimented. The supernatant is decanted and the whole process is repeated four to five times. The supernatant is then centrifuged for 1 minute at 1000 rpm and the collagenase solution is discarded. The remaining pellet is shaken with ice-cold HBBS and sedimented for ca. 10 minutes on ice. This wash process is repeated for a further three times. The faint pink stained islets are picked out from the washed pellets under a stereoscopic magnifying glass and transferred to culture medium (100 ml RPMI 1640 (Gibco), 1 ml glutamine, 1 ml penicillin, 1 ml Cibrobay antibiotic (Bayer), 10 ml foetal calf serum, 2 ml Hepes buffer 1 M). In order to obtain the purest possible culture, the islets are picked two to three times and transferred to fresh culture medium.

#### Stimulation of the islets

The islet cells from the culture medium are distributed in Eppendorff vessels containing 200 µl stimulation





buffer (118 mM NaCl, 0.2 mM NaH<sub>2</sub>PO<sub>4</sub>, 0.565 mM MgCl<sub>2</sub>, 1.25 mM CaCl<sub>2</sub>, 4.7 mM KCl, 10 mM Hepes, 1 % BSA, 3.3 mM glucose; pH 7.4) in a quantity of 10 islets per Eppendorff vessel and placed in an incubator for 1 hour at 37°C. Subsequently the peptides to be tested are added and filled up to 500 ml with stimulation buffer and incubated for one hour at 37°C. The islets are centrifuged at 1000 rpm for 1 minute. The amount of C-peptide is measured in the supernatant using an insulin-RIA (DPC Biermann, Nauheim). Each test substance was determined in quadruplicate.

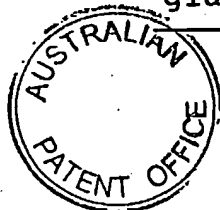
#### Activity of the exendin analogues

Some exendin analogues were tested as described above on isolated islet cells for insulin secretory activity. The data are shown as an example in the following table.

Insulin release from isolated islets after 1 hour  
[mIU/h/10 islets] in the presence of 10 mM glucose:

Table 6

	Control	GLP1-(7-36)-NH <sub>2</sub>	Seq.ID 84
10 mM glucose	30,21		
10 <sup>-7</sup> (10mM glucose)		53,52	48,94
10 <sup>-8</sup> (10mM glucose)		42,78	41,72
10 <sup>-9</sup> (10mM glucose)		29,99	38,76
10 <sup>-10</sup> (10mM glucose)			35,05



Measurement of the increase of the cytosolic calcium concentration in B cells of the endocrine pancreas (clonal B cell line INS-1)

**Culture of INS-1-cells (Asfari, M., 1992):**

INS-1 cells are cultured in RPMI 1640 medium containing 10 % FCS, 10 mM HEPES buffer (pH 7.4), 2 mM L-glutamine, 100 i.U. penicillin/ml, 100 µg streptomycin/ml, 1 mM pyruvate (sodium salt) and 50 µM 2-mercaptoethanol at 37°C in an atmosphere of 95 % air and 5 % CO<sub>2</sub>. After 6 to 8 days growth on plastic cell culture plates, the subconfluent cells, after rinsing once with PBS (phosphate-buffered saline), are detached from the base by incubating for four minutes at 37°C with 0.025 % trypsin and 0.27 mM EDTA in isoosmotic saline solution

**Preparation of the cells for calcium measurements:**

The detached cells are resuspended in Spinner medium (culture medium as above, but containing 5 % FCS and 25 mM HEPES) and incubated for two and a half hours in a Spinner bottle with a stirrer bar. Afterwards the medium is removed by centrifugation and the cells are resuspended in Spinner medium. They are then incubated for 30 minutes at 37°C with 2 µM Fura-2/acetoxymethyl ester under the same conditions as before. The Fura loading of the cells is terminated by washing the cells once with Spinner medium (room temperature). Afterwards the cells are resuspended in Spinner medium at room temperature ( $2 \times 10^7$  cells/ml). The cells are then removed from this suspension for calcium measurements.

**Measurements of the cytosolic calcium concentration:**

The measurements are carried out at 37°C in a modified Krebs-Ringer buffer (KRBH) containing 136 mM NaCl,



4.8 mM KCl, 2 mM CaCl<sub>2</sub>, 1.2 mM MgSO<sub>4</sub>, 1.2 mM KH<sub>2</sub>PO<sub>4</sub>, 5 mM NaHCO<sub>3</sub>, 10 mM glucose, 250 µM sulfinpyrazone (to inhibit the Fura-2 efflux into the medium) and 25 mM HEPES buffer (adjusted to pH 7.4 with NaOH). The cell concentration is 1-2 x 10<sup>6</sup>/ml. The measurements are carried out in a cuvette stirred with a stirring bar in a spectrofluorimeter at 37°C with 1.5 ml cell suspension. The excitation wavelength is 340 nm, the emission wavelength is 505 nm. At the end of the measurement 50 µM MnCl<sub>2</sub> is added which is followed by 100 µM DTPA (diethylenetriamine pentaacetate) in order to determine the proportion of extracellular fluorescence indicator relative to the measured fluorescence by temporarily quenching the fluorescence of extracellular Fura. After the addition of DTPA the entire Fura is firstly converted into a calcium-saturated and then into a calcium-free state to determine the calibration values F<sub>max</sub> (calcium-saturated) and F<sub>min</sub> (calcium-free) for the respective measurement. For this purpose the cells are lysed by adding 0.1 % Triton X-100. The dye is saturated with calcium by contact with the high extracellular calcium concentration. Subsequently 5 mM EGTA (ethylenedis(oxy-ethylenenitrilo)-tetraacetate) and 20 mM Tris solution are added in order to completely convert the dye into the calcium-free form.

The cytosolic calcium ion concentration is calculated according to the algorithm introduced by R. Tsien and colleagues (Grynkiewicz, G., 1985):

$$[Ca^{2+}]_{cyt} = ((F - F_{min}) / (F_{max} - F)) \times K_D$$

(F: fluorescence of the respective measurement point;  
K<sub>D</sub>: dissociation constant of the calcium complex of Fura-2, 225 nM (Grynkiewicz, G., 1985)).



(Before this calculation a compensation is carried out for the presence of extracellular Fura. For this the fluorescence quantity (extracellular Fura) determined by manganese addition is firstly subtracted from the fluorescence values of the measurement points. Then  $F_{\max}$  is corrected by subtracting this quantity. Finally the correction value for  $F_{\min}$  is determined. For this purpose the fluorescence quantity determined by addition of manganese is divided by the value 2.24. The value 2.24 was determined as the intrinsic instrument proportionality factor between the fluorescence of calcium-saturated and calcium-free Fura-2 at an excitation wavelength of 340 nm (measured with unesterified, free Fura-2). The correction value obtained in this manner was subtracted from  $F_{\min}$ ).

The examined peptides were added as 1000-fold concentrated solutions ( $10^{-5}$  M) in KRBH without  $\text{CaCl}_2$  and glucose.

#### Activity of the exendin analogues

Several exendin analogues were tested in the calcium assay on INS-1 cells as described above for their biological activity. The data are shown as an example in Figure 1 as well as in Table 7.



Table 7

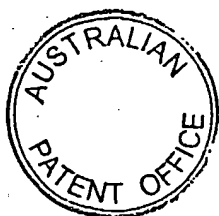
SEQ. ID Nr.	Concentration of the peptid s 10 <sup>-8</sup> M	$\Delta[\text{Ca}^{2+}]_{\text{cyt}}$ ± SD (n = 4)
6	[Arg <sup>30</sup> ]-exendin-(1-30)-NH <sub>2</sub>	64 ± 8 nM
3	[Nle <sup>14</sup> , Arg <sup>30</sup> ]-exendin-(1-30)- NH <sub>2</sub>	63 ± 8 nM
8	[Ala <sup>21</sup> , Arg <sup>30</sup> ]-exendin-(1-30)- NH <sub>2</sub>	61 ± 11 nM
9	[Ala <sup>28</sup> , Arg <sup>30</sup> ]-exendin-(1-30)- NH <sub>2</sub>	65 ± 15 nM
10	[Ala <sup>21,28</sup> , Arg <sup>30</sup> ]-exendin-(1- 30)- NH <sub>2</sub>	69 ± 30 nM
Control:		
	GLP-1-(7-36)amide	65 ± 10 nM

Competition with GLP-1-(7-36)-NH<sub>2</sub> on B cells of the endocrine pancreas (clonal B cell line INS-1)

Culture of INS-1-cells (Asfari, M., 1992)  
see measurement of calcium concentration

#### Competition experiments

The detached cells are taken up and suspended in Krebs-Ringer buffer (25 mM Tris, 120 mM NaCl, 1.2 mM MgSO<sub>4</sub>, 5 mM KCl, 1 mM Na-EDTA, 15 mM CH<sub>3</sub>COONa adjusted to pH 7.4 and supplemented with 1 % HSA and 0.1 % bacitracin). 250 ml is removed each time from this suspension for a reaction mixture, admixed with 20 ml tracer (<sup>125</sup>I-GLP1-(7-36)-NH<sub>2</sub>, 20,000 cpm) and 30 ml of the peptide to be examined in the corresponding dilution. Subsequently it is incubated for 30 minutes at 37°C, centrifuged for 4 minutes at 13,000 rpm, washed three times with buffer



and the radioactivity bound to the pellet ( $\gamma$ -counter) is measured. Competition curves were obtained by incubation with 10 different dilutions of the peptide to be tested ( $10^{-10}$ - $10^{-6}$  M in Krebs-Ringer buffer).

### Receptor affinity of the exendin analogues

The data are shown as an example in Table 8. GLP-1-(7-36)-NH<sub>2</sub> served as a standard.

Table 8

Seq. peptide ID	KdGLP1 $\pm$ SD [nM]	Kd $\pm$ SD [nM]	Kd/KdGLP1
69 [Nle <sup>14</sup> , Tyr <sup>30</sup> ]-Ex3-(1-30)-NH <sub>2</sub>	1,04 $\pm$ 0,05	0,56 $\pm$ 0,08	0,5

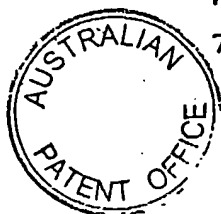
Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

The reference to any prior art in this specification is not, and should not be taken as, an acknowledgment or any form of suggestion that that prior art forms part of the common general knowledge in Australia.



Lit ratur

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SEQUENCE PROTOCOL

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(ii) TITLE OF INVENTION: Exendin analogues, processes for their production and pharmaceutical preparations containing them

(iii) NUMBER OF SEQUENCES: 118

(iv) COMPUTER READABLE FORM:

- (A) DATA CARRIER: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0, Version #1.30B (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(ix) CHARACTERISTICS:

- (A) NAME/KEY: peptide
- (B) POSITION: 30
- (D) OTHER INFORMATION: /product= "Xaa denotes all amino acids except for Gly"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

His	Ser	Asp	Gly	Thr	Phe	Thr	Ser	Asp	Leu	Ser	Lys	Gln	Met	Glu	Glu
1				5					10					15	
Glu	Ala	Val	Arg	Leu	Phe	Ile	Glu	Trp	Leu	Lys	Asn	Gly	Xaa		
			20					25					30		



(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(ix) CHARACTERISTICS:

- (A) NAME/KEY: peptide
- (B) POSITION: 30
- (D) OTHER INFORMATION: /product= "Xaa denotes all amino acids except for Gly"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

His	Gly	Glu	Gly	Thr	Phe	Thr	Ser	Asp	Leu	Ser	Lys	Gln	Met	Glu	Glu
1				5					10					15	
Glu	Ala	Val	Arg	Leu	Phe	Ile	Glu	Trp	Leu	Lys	Asn	Gly	Xaa		
			20					25					30		

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(ix) CHARACTERISTICS:

- (A) NAME/KEY: peptide
- (B) POSITION: 14
- (D) OTHER INFORMATION: /product= "Xaa denotes Nle"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

His	Gly	Glu	Gly	Thr	Phe	Thr	Ser	Asp	Leu	Ser	Lys	Gln	Xaa	Glu	Glu
1				5					10					15	
Glu	Ala	Val	Arg	Leu	Phe	Ile	Glu	Trp	Leu	Lys	Asn	Gly	Arg		
			20					25					30		



(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(ix) CHARACTERISTICS:

- (A) NAME/KEY: peptide
- (B) POSITION:14
- (D) OTHER INFORMATION:/product= "Xaa denotes Nle"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

His	Gly	Glu	Gly	Thr	Phe	Thr	Ser	Asp	Leu	Ser	Lys	Gln	Xaa	Glu	Glu
1				5					10					15	
Glu	Ala	Val	Arg	Leu	Phe	Ile	Glu	Trp	Leu	Lys	Asn	Gly	Tyr		
			20					25					30		

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(ix) CHARACTERISTICS:

- (A) NAME/KEY: peptide
- (B) POSITION:14
- (D) OTHER INFORMATION:/product= "Xaa denotes Nle"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

His	Ser	Asp	Gly	Thr	Phe	Thr	Ser	Asp	Leu	Ser	Lys	Gln	Xaa	Glu	Glu
1				5					10					15	
Glu	Ala	Val	Arg	Leu	Phe	Ile	Glu	Trp	Leu	Lys	Asn	Gly	Arg		
			20					25					30		



(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

His	Gly	Glu	Gly	Thr	Phe	Thr	Ser	Asp	Leu	Ser	Lys	Gln	Met	Glu	Glu
1				5					10					15	
Glu	Ala	Val	Arg	Leu	Phe	Ile	Glu	Trp	Leu	Lys	Asn	Gly	Arg		
			20					25					30		

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 29 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(ix) CHARACTERISTICS:

- (A) NAME/KEY: peptide
- (B) POSITION: 13
- (D) OTHER INFORMATION: /product= "Xaa denotes Nle"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Gly	Glu	Gly	Thr	Phe	Thr	Ser	Asp	Leu	Ser	Lys	Gln	Xaa	Glu	Glu	Glu
1				5					10					15	
Ala	Val	Arg	Leu	Phe	Ile	Glu	Trp	Leu	Lys	Asn	Gly	Arg			
			20					25							

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid



- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

His	Gly	Glu	Gly	Thr	Phe	Thr	Ser	Asp	Leu	Ser	Lys	Gln	Met	Glu	Glu
1				5					10					15	
Glu	Ala	Val	Arg	Ala	Phe	Ile	Glu	Trp	Leu	Lys	Asn	Gly	Arg		
			20					25					30		

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

His	Gly	Glu	Gly	Thr	Phe	Thr	Ser	Asp	Leu	Ser	Lys	Gln	Met	Glu	Glu
1				5					10					15	
Glu	Ala	Val	Arg	Leu	Phe	Ile	Glu	Trp	Leu	Lys	Ala	Gly	Arg		
			20					25					30		

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide



(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu  
1 5 10 15  
Glu Ala Val Arg Ala Phe Ile Glu Trp Leu Lys Ala Gly Arg  
20 25 30

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Ala Glu Glu  
1 5 10 15  
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Arg  
20 25 30

(2) INFORMATION FOR SEQ ID NO: 12:

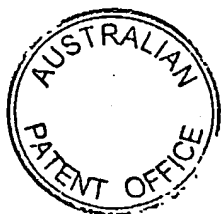
(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Ile Glu Glu  
1 5 10 15  
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Arg  
20 25 30



(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(ix) CHARACTERISTICS:

- (A) NAME/KEY: peptide
- (B) POSITION:14
- (D) OTHER INFORMATION:/product= "Xaa denotes Nle"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

His	Gly	Glu	Gly	Thr	Phe	Thr	Ser	Asp	Leu	Ser	Lys	Gln	Xaa	Glu	Glu
1				5					10					15	
Glu	Ala	Val	Arg	Leu	Phe	Ile	Glu	Trp	Leu	Lys					
			20					25							

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(ix) CHARACTERISTICS:

- (A) NAME/KEY: peptide
- (B) POSITION:14
- (D) OTHER INFORMATION:/product= "Xaa denotes Nle"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

His	Ser	Asp	Gly	Thr	Phe	Thr	Ser	Asp	Leu	Ser	Lys	Gln	Ala	Glu	Glu
1				5					10					15	
Glu	Ala	Val	Arg	Leu	Phe	Ile	Glu	Trp	Leu	Lys	Asn	Ala	Arg		
			20					25					30		



(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(ix) CHARACTERISTICS:

- (A) NAME/KEY: peptide
- (B) POSITION:14
- (D) OTHER INFORMATION:/product= "Xaa denotes Nle"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15

Glu	Ala	Val	Arg	Leu	Gly	Thr	Phe	Thr	Ser	Asp	Leu	Ser	Lys	Gln	Ala
1				5					10					15	
Glu	Glu	Glu	Trp	Leu	Ala	Asn	Gly	Arg							
			20				25							30	

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C)
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(ix) CHARACTERISTICS:

- (A) NAME/KEY: peptide
- (B) POSITION:14
- (D) OTHER INFORMATION:/product= "Xaa denotes Nle"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

His	Ser	Asp	Gly	Thr	Phe	Thr	Ser	Asp	Leu	Ser	Lys	Gln	Ala	Glu	Glu
1				5					10					15	
Glu	Ala	Val	Arg	Leu	Phe	Ile	Glu	Trp	Ala	Lys	Asn	Gly	Arg		
			20				25						30		





(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(ix) CHARACTERISTICS:

- (A) NAME/KEY: peptide
- (B) POSITION:14
- (D) OTHER INFORMATION:/product= "Xaa denotes Nle"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

His	Ala	Glu	Gly	Thr	Phe	Thr	Ser	Asp	Leu	Ser	Lys	Gln	Xaa	Glu	Glu
1				5					10					15	
Glu	Ala	Val	Arg	Leu	Phe	Ile	Glu	Trp	Leu	Lys	Asn	Gly	Arg		
			20					25					30		

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(ix) CHARACTERISTICS:

- (A) NAME/KEY: peptide
- (B) POSITION:14
- (D) OTHER INFORMATION:/product= "Xaa denotes Nle"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

His	Cys	Glu	Gly	Thr	Phe	Thr	Ser	Asp	Leu	Ser	Lys	Gln	Xaa	Glu	Glu
1				5					10					15	
Glu	Ala	Val	Arg	Leu	Phe	Ile	Glu	Trp	Leu	Lys	Asn	Gly	Arg		
			20					25					30		



(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(ix) CHARACTERISTICS:

- (A) NAME/KEY: peptide
- (B) POSITION: 14
- (D) OTHER INFORMATION: /product= "Xaa denotes Nle"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

His	Asp	Glu	Gly	Thr	Phe	Thr	Ser	Asp	Leu	Ser	Lys	Gln	Xaa	Glu	Glu
1				5					10					15	
Glu	Ala	Val	Arg	Leu	Phe	Ile	Glu	Trp	Leu	Lys	Asn	Gly	Arg		
			20					25					30		

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(ix) CHARACTERISTICS:

- (A) NAME/KEY: peptide
- (B) POSITION: 14
- (D) OTHER INFORMATION: /product= "Xaa denotes Nle"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

His	Glu	Glu	Gly	Thr	Phe	Thr	Ser	Asp	Leu	Ser	Lys	Gln	Xaa	Glu	Glu
1				5					10					15	
Glu	Ala	Val	Arg	Leu	Phe	Ile	Glu	Trp	Leu	Lys	Asn	Gly	Arg		
			20					25					30		



(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(ix) CHARACTERISTICS:

- (A) NAME/KEY: peptide
- (B) POSITION:14
- (D) OTHER INFORMATION:/product= "Xaa denotes Nle"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

His	Phe	Glu	Gly	Thr	Phe	Thr	Ser	Asp	Leu	Ser	Lys	Gln	Xaa	Glu	Glu
1				5					10					15	
Glu	Ala	Val	Arg	Leu	Phe	Ile	Glu	Trp	Leu	Lys	Asn	Gly	Arg		
			20					25					30		

(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

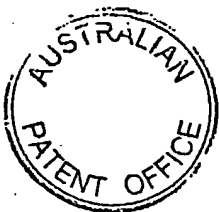
(ii) TYPE OF MOLECULE: peptide

(ix) CHARACTERISTICS:

- (A) NAME/KEY: peptide
- (B) POSITION:14
- (D) OTHER INFORMATION:/product= "Xaa denotes Nle"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

His	His	Glu	Gly	Thr	Phe	Thr	Ser	Asp	Leu	Ser	Lys	Gln	Xaa	Glu	Glu
1				5					10					15	
Glu	Ala	Val	Arg	Leu	Phe	Ile	Glu	Trp	Leu	Lys	Asn	Gly	Arg		
			20					25					30		



(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(ix) CHARACTERISTICS:

- (A) NAME/KEY: peptide
- (B) POSITION: 14
- (D) OTHER INFORMATION: /product= "Xaa denotes Nle"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

His	Ile	Glu	Gly	Thr	Phe	Thr	Ser	Asp	Leu	Ser	Lys	Gln	Xaa	Glu	Glu
1				5					10					15	
Glu	Ala	Val	Arg	Leu	Phe	Ile	Glu	Trp	Leu	Lys	Asn	Gly	Arg		
			20					25					30		

(2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(ix) CHARACTERISTICS:

- (A) NAME/KEY: peptide
- (B) POSITION: 14
- (D) OTHER INFORMATION: /product= "Xaa denotes Nle"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

His	Lys	Glu	Gly	Thr	Phe	Thr	Ser	Asp	Leu	Ser	Lys	Gln	Xaa	Glu	Glu
1				5					10					15	
Glu	Ala	Val	Arg	Leu	Phe	Ile	Glu	Trp	Leu	Lys	Asn	Gly	Arg		
			20					25					30		



(2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(ix) CHARACTERISTICS:

- (A) NAME/KEY: peptide
- (B) POSITION: 14
- (D) OTHER INFORMATION: /product= "Xaa denotes Nle"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

His	Leu	Glu	Gly	Thr	Phe	Thr	Ser	Asp	Leu	Ser	Lys	Gln	Xaa	Glu	Glu
1				5					10					15	
Glu	Ala	Val	Arg	Leu	Phe	Ile	Glu	Trp	Leu	Lys	Asn	Gly	Arg		
			20					25					30		

(2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(ix) CHARACTERISTICS:

- (A) NAME/KEY: peptide
- (B) POSITION: 14
- (D) OTHER INFORMATION: /product= "Xaa denotes Nle"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

His	Met	Glu	Gly	Thr	Phe	Thr	Ser	Asp	Leu	Ser	Lys	Gln	Xaa	Glu	Glu
1				5					10					15	
Glu	Ala	Val	Arg	Leu	Phe	Ile	Glu	Trp	Leu	Lys	Asn	Gly	Arg		
			20					25					30		



(2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(ix) CHARACTERISTICS:

- (A) NAME/KEY: peptide
- (B) POSITION:14
- (D) OTHER INFORMATION:/product= "Xaa denotes Nle"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

His	Asn	Glu	Gly	Thr	Phe	Thr	Ser	Asp	Leu	Ser	Lys	Gln	Xaa	Glu	Glu
1				5					10					15	
Glu	Ala	Val	Arg	Leu	Phe	Ile	Glu	Trp	Leu	Lys	Asn	Gly	Arg		
			20				25						30		

(2) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

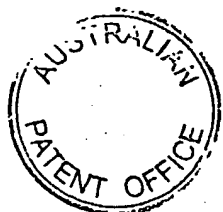
(ii) TYPE OF MOLECULE: peptide

(ix) CHARACTERISTICS:

- (A) NAME/KEY: peptide
- (B) POSITION:14
- (D) OTHER INFORMATION:/product= "Xaa denotes Nle"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

His	Pro	Glu	Gly	Thr	Phe	Thr	Ser	Asp	Leu	Ser	Lys	Gln	Xaa	Glu	Glu
1				5					10					15	
Glu	Ala	Val	Arg	Leu	Phe	Ile	Glu	Trp	Leu	Lys	Asn	Gly	Arg		
			20				25						30		



(2) INFORMATION FOR SEQ ID NO: 29:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(ix) CHARACTERISTICS:

- (A) NAME/KEY: peptide
- (B) POSITION:14
- (D) OTHER INFORMATION:/product= "Xaa denotes Nle"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

His	Gln	Glu	Gly	Thr	Phe	Thr	Ser	Asp	Leu	Ser	Lys	Gln	Xaa	Glu	Glu
1				5					10					15	
Glu	Ala	Val	Arg	Leu	Phe	Ile	Glu	Trp	Leu	Lys	Asn	Gly	Arg		
			20					25					30		

(2) INFORMATION FOR SEQ ID NO: 30:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(ix) CHARACTERISTICS:

- (A) NAME/KEY: peptide
- (B) POSITION:14
- (D) OTHER INFORMATION:/product= "Xaa denotes Nle"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

His	Arg	Glu	Gly	Thr	Phe	Thr	Ser	Asp	Leu	Ser	Lys	Gln	Xaa	Glu	Glu
1				5					10					15	
Glu	Ala	Val	Arg	Leu	Phe	Ile	Glu	Trp	Leu	Lys	Asn	Gly	Arg		
			20					25					30		



(2) INFORMATION FOR SEQ ID NO: 31:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(ix) CHARACTERISTICS:

- (A) NAME/KEY: peptide
- (B) POSITION:14
- (D) OTHER INFORMATION:/product= "Xaa denotes Nle"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

His Ser Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Xaa Glu Glu  
1                      5                      10                      15  
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Arg  
                    20                      25                      30

(2) INFORMATION FOR SEQ ID NO: 32:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

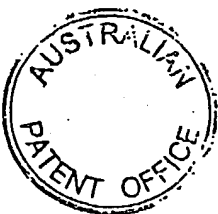
(ii) TYPE OF MOLECULE: peptide

(ix) CHARACTERISTICS:

- (A) NAME/KEY: peptide
- (B) POSITION:14
- (D) OTHER INFORMATION:/product= "Xaa denotes Nle"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

His Thr Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Xaa Glu Glu  
1                      5                      10                      15  
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Arg  
                    20                      25                      30





(2) INFORMATION FOR SEQ ID NO: 33:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(ix) CHARACTERISTICS:

- (A) NAME/KEY: peptide
- (B) POSITION:14
- (D) OTHER INFORMATION:/product= "Xaa denotes Nle"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

His	Val	Glu	Gly	Thr	Phe	Thr	Ser	Asp	Leu	Ser	Lys	Gln	Xaa	Glu	Glu
1				5					10					15	
Glu	Ala	Val	Arg	Leu	Phe	Ile	Glu	Trp	Leu	Lys	Asn	Gly	Arg		
			20					25					30		

(2) INFORMATION FOR SEQ ID NO: 34:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(ix) CHARACTERISTICS:

- (A) NAME/KEY: peptide
- (B) POSITION:14
- (D) OTHER INFORMATION:/product= "Xaa denotes Nle"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:

His	Trp	Glu	Gly	Thr	Phe	Thr	Ser	Asp	Leu	Ser	Lys	Gln	Xaa	Glu	Glu
1				5					10					15	
Glu	Ala	Val	Arg	Leu	Phe	Ile	Glu	Trp	Leu	Lys	Asn	Gly	Arg		
			20					25					30		



(2) INFORMATION FOR SEQ ID NO: 35:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(ix) CHARACTERISTICS:

- (A) NAME/KEY: peptide
- (B) POSITION: 14
- (D) OTHER INFORMATION: /product= "Xaa denotes Nle"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

His	Tyr	Glu	Gly	Thr	Phe	Thr	Ser	Asp	Leu	Ser	Lys	Gln	Xaa	Glu	Glu
1				5					10					15	
Glu	Ala	Val	Arg	Leu	Phe	Ile	Glu	Trp	Leu	Lys	Asn	Gly	Arg		
			20					25					30		

(2) INFORMATION FOR SEQ ID NO: 36:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36:

His	Ala	Asp	Gly	Thr	Phe	Thr	Ser	Asp	Leu	Ser	Ser	Tyr	Met	Glu	Gly
1				5					10					15	
Gln	Ala	Val	Lys	Glu	Phe	Ile	Ala	Trp	Leu	Val	Lys	Gly	Arg		
			20					25					30		

(2) INFORMATION FOR SEQ ID NO: 37:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand



(D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 37:

His Ser Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Ala Glu Glu  
1 5 10 15  
Glu Ala Val Arg Leu Phe Ile Glu Ala Leu Lys Asn Gly Arg  
20 25 30

(2) INFORMATION FOR SEQ ID NO: 38:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 38:

His Ser Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Ala Glu Glu  
1 5 10 15  
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Arg  
20 25 30

(2) INFORMATION FOR SEQ ID NO: 39:

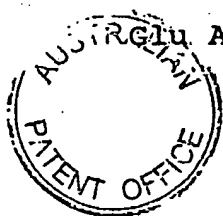
(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 39:

Ala Ser Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Val Glu Glu  
1 5 10 15  
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Arg  
20 25 30



(2) INFORMATION FOR SEQ ID NO: 40:

- (i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 30 amino acids  
    (B) TYPE: amino acid  
    (C) STRANDEDNESS: single strand  
    (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 40:

His	Gly	Ala	Gly	Thr	Phe	Thr	Ser	Asp	Leu	Ser	Lys	Gln	Ala	Glu	Glu	
1				5					10					15		
Glu	Ala	Val	Arg	Leu	Phe	Ile	Glu	Trp	Leu	Lys	Asn	Gly	Arg			
			20					25					30			

INFORMATION FOR SEQ ID NO: 41:

- (i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 30 amino acids  
    (B) TYPE: amino acid  
    (C) STRANDEDNESS: single strand  
    (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 41:

His	Gly	Glu	Gly	Ala	Phe	Thr	Ser	Asp	Leu	Ser	Lys	Gln	Ala	Glu	Glu	
1				5					10					15		
Glu	Ala	Val	Arg	Leu	Phe	Ile	Glu	Trp	Leu	Lys	Asn	Gly	Arg			
			20					25					30			

(2) INFORMATION FOR SEQ ID NO: 42:

- (i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 30 amino acids  
    (B) TYPE: amino acid  
    (C) STRANDEDNESS: single strand  
    (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide



(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 42:

His Gly Glu Gly Tyr Phe Thr Ser Asp Leu Ser Lys Gln Ala Glu Glu  
1 5 10 15

Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Arg  
20 25 30

(2) INFORMATION FOR SEQ ID NO: 43:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single strand  
(D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 43:

His Gly Glu Gly Thr Tyr Thr Ser Asp Leu Ser Lys Gln Ala Glu Glu  
1 5 10 15

Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Arg  
20 25 30

(2) INFORMATION FOR SEQ ID NO: 44:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single strand  
(D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 44:

His Gly Glu Gly Thr Ile Thr Ser Asp Leu Ser Lys Gln Ala Glu Glu  
1 5 10 15

Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Arg  
20 25 30



(2) INFORMATION FOR SEQ ID NO: 45:

(i) SEQUENCE CHARACTERISTICS:

- SEQUENCE CHARACTERISTICS
- (A) LENGTH: 30 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single strand
  - (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 45:

His Gly Glu Gly Thr Phe Ser Ser Asp Leu Ser Lys Gln Ala Glu Glu  
1 5 10 15  
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Arg  
20 25 30

(2) INFORMATION FOR SEQ ID NO: 46:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single strand  
(D) TOPOLOGY: linear

## (ii) TYPE OF MOLECULE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 46:

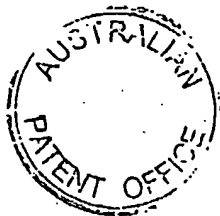
His Gly Glu Gly Thr Phe Tyr Ser Asp Leu Ser Lys Gln Ala Glu Glu  
1 5 10 15  
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Arg  
20 25 30

(2) INFORMATION FOR SEQ ID NO: 47:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single strand  
(D) TOPOLOGY: linear

## (ii) TYPE OF MOLECULE: peptide



(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 47:

His Gly Glu Gly Thr Phe Thr Thr Asp Leu Ser Lys Gln Ala Glu Glu  
1 5 10 15  
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Arg  
20 25 30

(2) INFORMATION FOR SEQ ID NO: 48:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 48:

His Gly Glu Gly Thr Phe Thr Tyr Asp Leu Ser Lys Gln Ala Glu Glu  
1 5 10 15  
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Arg  
20 25 30

(2) INFORMATION FOR SEQ ID NO: 49:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 49:

His Gly Glu Gly Thr Phe Thr Ser Glu Leu Ser Lys Gln Ala Glu Glu  
1 5 10 15  
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Arg  
20 25 30



(2) INFORMATION FOR SEQ ID NO: 50:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 50:

His Gly Glu Gly Thr Phe Thr Ser Asp Ala Ser Lys Gln Ala Glu Glu  
1                      5                      10                      15  
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Arg  
                    20                      25                      30

(2) INFORMATION FOR SEQ ID NO: 51:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 51:

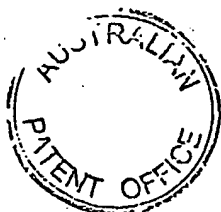
His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ala Lys Gln Ala Glu Glu  
1                      5                      10                      15  
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Arg  
                    20                      25                      30

(2) INFORMATION FOR SEQ ID NO: 52:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide





(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 52:

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Ala Gln Ala Glu Glu  
1 5 10 15  
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Arg  
20 25 30

(2) INFORMATION FOR SEQ ID NO: 53:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single strand  
(D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 53:

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Ala Ala Glu Glu  
1 5 10 15  
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Arg  
20 25 30

(2) INFORMATION FOR SEQ ID NO: 54:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single strand  
(D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 54:

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Ala Ala Glu  
1 5 10 15  
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Arg  
20 25 30



(2) INFORMATION FOR SEQ ID NO: 55:

- (i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 30 amino acids  
    (B) TYPE: amino acid  
    (C) STRANDEDNESS: single strand  
    (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 55:

Gly	Ser	Asp	Gly	Ser	Phe	Thr	Ser	Asp	Leu	Ser	Lys	Gln	Ala	Glu	Ala
1				5					10					15	
Glu	Ala	Val	Arg	Leu	Phe	Ile	Glu	Trp	Leu	Lys	Asn	Gly	Arg		
			20					25					30		

(2) INFORMATION FOR SEQ ID NO: 56:

- (i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 30 amino acids  
    (B) TYPE: amino acid  
    (C) STRANDEDNESS: single strand  
    (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 56:

Lys	Gly	Glu	Gly	Thr	Phe	Thr	Ser	Asp	Leu	Ser	Lys	Gln	Ala	Glu	Glu
1				5					10					15	
Ala	Ala	Val	Arg	Leu	Phe	Ile	Glu	Trp	Leu	Lys	Asn	Gly	Arg		
			20					25					30		

(2) INFORMATION FOR SEQ ID NO: 57:

- (i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 30 amino acids  
    (B) TYPE: amino acid  
    (C) STRANDEDNESS: single strand  
    (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide



(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 57:

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Ala Glu Glu  
1 5 10 15  
Glu Leu Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Arg  
20 25 30

(2) INFORMATION FOR SEQ ID NO: 58:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 58:

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Ala Glu Glu  
1 5 10 15  
Glu Ala Ile Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Arg  
20 25 30

(2) INFORMATION FOR SEQ ID NO: 59:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 59:

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Ala Glu Glu  
1 5 10 15  
Glu Ala Val Ala Leu Phe Ile Glu Trp Leu Lys Asn Gly Arg  
20 25 30



(2) INFORMATION FOR SEQ ID NO: 60:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 60:

His	Ser	Asp	Gly	Thr	Phe	Thr	Ser	Asp	Leu	Ser	Lys	Gln	Ala	Glu	Glu
1				5					10					15	
Glu	Ala	Val	Arg	Leu	Tyr	Ile	Glu	Trp	Leu	Lys	Asn	Gly	Arg		
			20					25					30		

(2) INFORMATION FOR SEQ ID NO: 61:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 61:

His	Gly	Glu	Gly	Thr	Phe	Thr	Ser	Asp	Leu	Ser	Lys	Gln	Ala	Glu	Glu
1				5					10					15	
Glu	Ala	Val	Arg	Leu	Phe	Val	Glu	Trp	Leu	Lys	Asn	Gly	Arg		
			20					25					30		

(2) INFORMATION FOR SEQ ID NO: 62:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide



(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 62:

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Ala Glu Glu  
1 5 10 15

Glu Ala Val Arg Leu Phe Ile Leu Trp Leu Lys Asn Gly Arg  
20 25 30

(2) INFORMATION FOR SEQ ID NO: 63:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 63:

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Ala Glu Glu  
1 5 10 15

Glu Ala Val Arg Leu Phe Ile Glu Ala Leu Lys Asn Gly Arg  
20 25 30

(2) INFORMATION FOR SEQ ID NO: 64:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 64:

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Ala Glu Glu  
1 5 10 15

Glu Ala Val Arg Leu Phe Ile Glu Trp Ala Lys Asn Gly Arg  
20 25 30



(2) INFORMATION FOR SEQ ID NO: 65:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single strand  
(D) TOPOLOGY: linear

## (ii) TYPE OF MOLECULE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 65:

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Ala Glu Glu  
1 5 10 15

Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Ala Asn Gly Arg  
20 25 30

(2) INFORMATION FOR SEQ ID NO: 66:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single strand  
(D) TOPOLOGY: linear

## (ii) TYPE OF MOLECULE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 66:

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Ala Glu Glu  
1 5 10 15

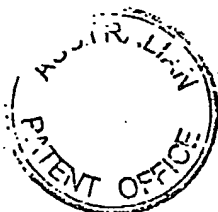
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Ala Arg  
20 25 30

(2) INFORMATION FOR SEQ ID NO: 67:

**(i) SEQUENCE CHARACTERISTICS:**

- (A) LENGTH: 30 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single strand  
(D) TOPOLOGY: linear

## (ii) TYPE OF MOLECULE: peptide





(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 69:

His	Ser	Asp	Gly	Thr	Phe	Thr	Ser	Asp	Leu	Ser	Lys	Gln	Xaa	Glu	Glu
1				5					10					15	
Glu	Ala	Val	Arg	Leu	Phe	Ile	Glu	Trp	Leu	Lys	Asn	Gly	Tyr		
			20					25					30		

(2) INFORMATION FOR SEQ ID NO: 70:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(ix) CHARACTERISTICS:

- (A) NAME/KEY: peptide
- (B) POSITION: 14
- (D) OTHER INFORMATION: /product= "Xaa denotes Nle"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 70:

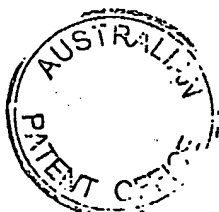
His	Ser	Asp	Gly	Thr	Phe	Thr	Ser	Asp	Leu	Ser	Lys	Gln	Xaa	Glu	Glu
1				5					10					15	
Glu	Ala	Val	Arg	Leu	Phe	Ile	Glu	Trp	Leu	Lys	Asn	Gly	Arg		
			20					25					30		

(2) INFORMATION FOR SEQ ID NO: 71:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide





(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 71:

His Ser Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Ala Glu Glu  
1 5 10 15  
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Arg  
20 25 30

(2) INFORMATION FOR SEQ ID NO: 72:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 29 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(ix) CHARACTERISTICS:

- (A) NAME/KEY: peptide
- (B) POSITION:13
- (D) OTHER INFORMATION:/product= "Xaa denotes Nle"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 72:

Ser Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Xaa Glu Glu Glu  
1 5 10 15  
Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Arg  
20 25

(2) INFORMATION FOR SEQ ID NO: 73:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 28 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(ix) CHARACTERISTICS:

- (A) NAME/KEY: peptide
- (B) POSITION:12
- (D) OTHER INFORMATION:/product= "Xaa denotes Nle"



(xi) SEQUENCE DESCRIPTION SEQ ID NO: 73:

Asp	Gly	Thr	Phe	Thr	Ser	Asp	Leu	Ser	Lys	Gln	Xaa	Glu	Glu	Glu	Ala
1				5					10					15	
Val	Arg	Leu	Phe	Ile	Glu	Trp	Leu	Lys	Asn	Gly	Arg				
			20					25							

(2) INFORMATION FOR SEQ ID NO: 74:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 29 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(ix) CHARACTERISTICS:

- (A) NAME/KEY: peptide
- (B) POSITION: 13
- (D) OTHER INFORMATION: /product= "Xaa denotes Nle"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 74:

Ser	Asp	Gly	Thr	Phe	Thr	Ser	Asp	Leu	Ser	Lys	Gln	Xaa	Glu	Glu	Glu
1				5					10					15	
Ala	Val	Arg	Leu	Phe	Ile	Glu	Trp	Leu	Lys	Asn	Gly	Arg			
			20					25							

(2) INFORMATION FOR SEQ ID NO: 75:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 28 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide



(ix) CHARACTERISTICS:

(A) NAME/KEY: peptide

(B) POSITION:12

(D) OTHER INFORMATION:/product= "Xaa denotes Nle"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 75:

Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Xaa Glu Glu Ala  
1 5 10 15

Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Arg  
20 25

(2) INFORMATION FOR SEQ ID NO: 76:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single strand

(D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(ix) CHARACTERISTICS:

(A) NAME/KEY: peptide

(B) POSITION:14

(D) OTHER INFORMATION:/product= "Xaa denotes Nle"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 76:

His Ser Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Xaa Glu Glu  
1 5 10 15

Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys  
20 25

(2) INFORMATION FOR SEQ ID NO: 77:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single strand

(D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide



(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 77:

His Lys Pro Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Ala Glu Glu  
1 5 10 15  
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Arg  
20 25 30

(2) INFORMATION FOR SEQ ID NO: 78:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single strand  
(D) TOPOLOGY: linear

## (ii) TYPE OF MOLECULE: peptide

(ix) CHARACTERISTICS:

- (A) NAME/KEY: peptide  
(B) POSITION:14  
(D) OTHER INFORMATION:/product= "Xaa denotes Nle"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 78:

His Ala Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Xaa Glu Glu  
1 5 10 15  
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Arg  
20 25 30

(2) INFORMATION FOR SEQ ID NO: 79:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single strand  
(D) TOPOLOGY: linear

## (ii) TYPE OF MOLECULE: peptide

(ix) CHARACTERISTICS:

- (A) NAME/KEY: peptide  
(B) POSITION:14  
(D) OTHER INFORMATION:/product= "Xaa denotes Nle"







(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 83:

His Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Xaa Glu Glu  
1 5 10 15

Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Arg  
20 25 30

(2) INFORMATION FOR SEQ ID NO: 84:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(ix) CHARACTERISTICS:

- (A) NAME/KEY: peptide
- (B) POSITION: 14
- (D) OTHER INFORMATION: /product= "Xaa denotes Nle"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 84:

His His Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Xaa Glu Glu  
1 5 10 15

Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Arg  
20 25 30

(2) INFORMATION FOR SEQ ID NO: 85:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(ix) CHARACTERISTICS:

- (A) NAME/KEY: peptide
- (B) POSITION: 14
- (D) OTHER INFORMATION: /product= "Xaa denotes Nle"



(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 85:

His Ile Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Xaa Glu Glu  
1                      5                      10                      15  
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Arg  
                    20                      25                      30

(2) INFORMATION FOR SEQ ID NO: 86:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(ix) CHARACTERISTICS:

- (A) NAME/KEY: peptide
- (B) POSITION: 14
- (D) OTHER INFORMATION: /product= "Xaa denotes Nle"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 86:

His Lys Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Xaa Glu Glu  
1                      5                      10                      15  
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Arg  
                    20                      25                      30

(2) INFORMATION FOR SEQ ID NO: 87:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(ix) CHARACTERISTICS:

- (A) NAME/KEY: peptide
- (B) POSITION: 14
- (D) OTHER INFORMATION: /product= "Xaa denotes Nle"





(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 87:

His	Leu	Asp	Gly	Thr	Phe	Thr	Ser	Asp	Leu	Ser	Lys	Gln	Xaa	Glu	Glu
1				5					10					15	
Glu	Ala	Val	Arg	Leu	Phe	Ile	Glu	Trp	Leu	Lys	Asn	Gly	Arg		
			20					25					30		

(2) INFORMATION FOR SEQ ID NO: 88:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(ix) CHARACTERISTICS:

- (A) NAME/KEY: peptide
- (B) POSITION: 14
- (D) OTHER INFORMATION: /product= "Xaa denotes Nle"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 88:

His	Met	Asp	Gly	Thr	Phe	Thr	Ser	Asp	Leu	Ser	Lys	Gln	Xaa	Glu	Glu
1				5					10					15	
Glu	Ala	Val	Arg	Leu	Phe	Ile	Glu	Trp	Leu	Lys	Asn	Gly	Arg		
			20					25					30		

(2) INFORMATION FOR SEQ ID NO: 89:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(ix) CHARACTERISTICS:

- (A) NAME/KEY: peptide
- (B) POSITION: 14
- (D) OTHER INFORMATION: /product= "Xaa denotes Nle"



(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 89:

His Asn Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Xaa Glu Glu  
1                      5                      10                      15  
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Arg  
                    20                      25                      30

(2) INFORMATION FOR SEQ ID NO: 90:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(ix) CHARACTERISTICS:

- (A) NAME/KEY: peptide
- (B) POSITION: 14
- (D) OTHER INFORMATION: /product= "Xaa denotes Nle"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 90:

His Pro Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Xaa Glu Glu  
1                      5                      10                      15  
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Arg  
                    20                      25                      30

(2) INFORMATION FOR SEQ ID NO: 91:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(ix) CHARACTERISTICS:

- (A) NAME/KEY: peptide
- (B) POSITION: 14
- (D) OTHER INFORMATION: /product= "Xaa denotes Nle"



(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 91:

His Gln Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Xaa Glu Glu  
1 5 10 15  
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Arg  
20 25 30

(2) INFORMATION FOR SEQ ID NO: 92:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(ix) CHARACTERISTICS:

- (A) NAME/KEY: peptide
- (B) POSITION:14
- (D) OTHER INFORMATION:/product= "Xaa denotes Nle"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 92:

His Arg Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Xaa Glu Glu  
1 5 10 15  
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Arg  
20 25 30

(2) INFORMATION FOR SEQ ID NO: 93:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(ix) CHARACTERISTICS:

- (A) NAME/KEY: peptide
- (B) POSITION:14
- (D) OTHER INFORMATION:/product= "Xaa denotes Nle"



(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 93:

His Thr Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Xaa Glu Glu  
1 5 10 15  
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Arg  
20 25 30

(2) INFORMATION FOR SEQ ID NO: 94:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single strand  
(D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(ix) CHARACTERISTICS:

- (A) NAME/KEY: peptide  
(B) POSITION:14  
(D) OTHER INFORMATION:/product= "Xaa denotes Nle"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 94:

His Val Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Xaa Glu Glu  
1 5 10 15  
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Arg  
20 25 30

(2) INFORMATION FOR SEQ ID NO: 95:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single strand  
(D) TOPOLOGY: linear

## (ii) TYPE OF MOLECULE: peptide

(ix) CHARACTERISTICS:

- (A) NAME/KEY: peptide  
(B) POSITION:14  
(D) OTHER INFORMATION:/product= "Xaa denotes Nle"



(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 95:

His	Trp	Asp	Gly	Thr	Phe	Thr	Ser	Asp	Leu	Ser	Lys	Gln	Xaa	Glu	Glu
1				5					10					15	
Glu	Ala	Val	Arg	Leu	Phe	Ile	Glu	Trp	Leu	Lys	Asn	Gly	Arg		
			20					25					30		

(2) INFORMATION FOR SEQ ID NO: 96:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(ix) CHARACTERISTICS:

- (A) NAME/KEY: peptide
- (B) POSITION: 14
- (D) OTHER INFORMATION: /product= "Xaa denotes Nle"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 96:

His	Tyr	Asp	Gly	Thr	Phe	Thr	Ser	Asp	Leu	Ser	Lys	Gln	Xaa	Glu	Glu
1				5					10					15	
Glu	Ala	Val	Arg	Leu	Phe	Ile	Glu	Trp	Leu	Lys	Asn	Gly	Arg		
			20					25					30		

(2) INFORMATION FOR SEQ ID NO: 97:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide



(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 97:

His Ser Ala Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Ala Glu Glu  
1                      5                      10                      15  
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Arg  
                    20                      25                      30

(2) INFORMATION FOR SEQ ID NO: 98:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 98:

His Ser Asp Gly Ala Phe Thr Ser Asp Leu Ser Lys Gln Ala Glu Glu  
1                      5                      10                      15  
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Arg  
                    20                      25                      30

(2) INFORMATION FOR SEQ ID NO: 99:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 99:

His Ser Asp Gly Tyr Phe Thr Ser Asp Leu Ser Lys Gln Ala Glu Glu  
1                      5                      10                      15  
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Arg  
                    20                      25                      30



(2) INFORMATION FOR SEQ ID NO: 100:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single strand  
(D) TOPOLOGY: linear

## (ii) TYPE OF MOLECULE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 100:

His Ser Asp Gly Thr Tyr Thr Ser Asp Leu Ser Lys Gln Ala Glu Glu  
1 5 10 15

Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Arg  
20 25 30

(2) INFORMATION FOR SEQ ID NO: 101:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single strand  
(D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 101:

His Ser Asp Gly Thr Ile Thr Ser Asp Leu Ser Lys Gln Ala Glu Glu  
1 5 10 15

Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Arg  
20 25 30

(2) INFORMATION FOR SEQ ID NO: 102:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single strand  
(D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide



(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 102:

His Ser Asp Gly Thr Phe Ser Ser Asp Leu Ser Lys Gln Ala Glu Glu  
1 5 10 15  
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Arg  
20 25 30

(2) INFORMATION FOR SEQ ID NO: 103:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 103:

His Ser Asp Gly Thr Phe Tyr Ser Asp Leu Ser Lys Gln Ala Glu Glu  
1 5 10 15  
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Arg  
20 25 30

(2) INFORMATION FOR SEQ ID NO: 104:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 104:

His Ser Asp Gly Thr Phe Thr Thr Asp Leu Ser Lys Gln Ala Glu Glu  
1 5 10 15  
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Arg  
20 25 30





(2) INFORMATION FOR SEQ ID NO: 105:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single strand  
(D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 105:

His	Ser	Asp	Gly	Thr	Phe	Thr	Tyr	Asp	Leu	Ser	Lys	Gln	Ala	Glu	Glu
1				5					10					15	
Glu	Ala	Val	Arg	Leu	Phe	Ile	Glu	Trp	Leu	Lys	Asn	Gly	Arg		
			20					25					30		

(2) INFORMATION FOR SEQ ID NO: 106:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single strand  
(D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

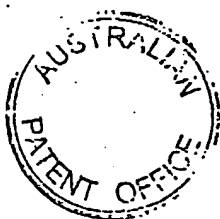
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 106:

His	Ser	Asp	Gly	Thr	Phe	Thr	Ser	Glu	Leu	Ser	Lys	Gln	Ala	Glu	Glu
1				5					10					15	
Glu	Ala	Val	Arg	Leu	Phe	Ile	Glu	Trp	Leu	Lys	Asn	Gly	Arg		
			20					25					30		

(2) INFORMATION FOR SEQ ID NO: 107:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single strand  
(D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide



(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 107:

His Ser Asp Gly Thr Phe Thr Ser Asp Ala Ser Lys Gln Ala Glu Glu  
1 5 10 15

Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Arg  
20 25 30

(2) INFORMATION FOR SEQ ID NO: 108:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single strand  
(D) TOPOLOGY: linear

## (ii) TYPE OF MOLECULE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 108:

His Ser Asp Gly Thr Phe Thr Ser Asp Leu Ala Lys Gln Ala Glu Glu  
1 5 10 15

Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Arg  
20 25 30

(2) INFORMATION FOR SEQ ID NO: 109:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single strand  
(D) TOPOLOGY: linear

## (ii) TYPE OF MOLECULE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 109:

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Ala Gln Ala Glu Glu  
1                      5                      10                      15

Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Arg  
20 25 30



(2) INFORMATION FOR SEQ ID NO: 110:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 110:

His	Ser	Asp	Gly	Thr	Phe	Thr	Ser	Asp	Leu	Ser	Lys	Ala	Ala	Glu	Glu
1				5					10					15	
Glu	Ala	Val	Arg	Leu	Phe	Ile	Glu	Trp	Leu	Lys	Asn	Gly	Arg		
			20					25					30		

(2) INFORMATION FOR SEQ ID NO: 111:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 111:

His	Ser	Asp	Gly	Thr	Phe	Thr	Ser	Asp	Leu	Ser	Lys	Gln	Ala	Ala	Glu
1				5					10					15	
Glu	Ala	Val	Arg	Leu	Phe	Ile	Glu	Trp	Leu	Lys	Asn	Gly	Arg		
			20					25					30		

(2) INFORMATION FOR SEQ ID NO: 112:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide



(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 112:

Lys Ser Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Ala Glu Glu  
1 5 10 15  
Ala Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Arg  
20 25 30

(2) INFORMATION FOR SEQ ID NO: 113:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 113:

His Ser Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Ala Glu Glu  
1 5 10 15  
Glu Leu Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Arg  
20 25 30

(2) INFORMATION FOR SEQ ID NO: 114:

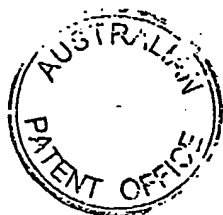
(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single strand
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 114:

His Ser Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Ala Glu Glu  
1 5 10 15  
Glu Ala Ile Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Arg  
20 25 30



(2) INFORMATION FOR SEQ ID NO: 115:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single strand  
(D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 115:

His Ser Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Ala Glu Glu  
1 5 10 15  
Glu Ala Val Ala Leu Phe Ile Glu Trp Leu Lys Asn Gly Arg  
20 25 30

(2) INFORMATION FOR SEQ ID NO: 116:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single strand  
(D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 116:

His Ser Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Ala Glu Glu  
1 5 10 15  
Glu Ala Val Arg Leu Tyr Ile Glu Trp Leu Lys Asn Gly Arg  
20 25 30

(2) INFORMATION FOR SEQ ID NO: 117:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single strand  
(D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: p ptide



(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 117:

His Ser Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Ala Glu Glu  
1 5 10 15  
Glu Ala Val Arg Leu Phe Val Glu Trp Leu Lys Asn Gly Arg  
20 25 30

(2) INFORMATION FOR SEQ ID NO: 118:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single strand  
(D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 118:

His Ser Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Ala Glu Glu  
1 5 10 15  
Glu Ala Val Arg Leu Phe Ile Leu Trp Leu Lys Asn Gly Arg  
20 25 30



THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. Peptide, wherein it has the sequence 1 or 2

SEQ ID NO:1

5  
1 5 10 15  
H S D G T F T S D L S K Q M E E E A V  
20 25 30  
R L F I E W L K N G X

10 SEQ ID NO: 2

1 5 10 15  
H G E G T F T S D L S K Q M E E E A V  
20 25 30  
15 R L F I E W L K N G X

in which X denotes one of the 20 proteinogenic amino acids from Table 1 apart from glycine or a non-proteinogenic, the amino acids in position 1, 2, 28, 29 or 30 can be part of the sequence depending on the desired chain length and the N-terminus is represented by

20  $NR_1R_2$  in which

$R_1$  denotes hydrogen, acetyl, trifluoroacetyl, adamantyl, Fmoc, Z, Boc, Alloc,  $C_1$ - $C_6$  alkyl, as hereinbefore defined,  $C_2$ - $C_8$  alkenyl or  $C_7$ - $C_9$  aralkyl,

$R_2$  denotes hydrogen, acetyl, trifluoroacetyl, adamantyl, Fmoc, Z, Boc, Alloc,  $C_4$ - $C_6$  alkyl, as hereinbefore defined,

25  $C_2$ - $C_8$  alkenyl or  $C_7$ - $C_9$  aralkyl,

and the C-terminus is represented by  $COR_3$  in which  $R_3$  equals  $OR_4$  or  $NR_4R_5$

in which  $R_4$  equals hydrogen or  $C_1$ - $C_6$  alkyl, as hereinbefore defined,

in which  $R_5$  equals hydrogen or  $C_1$ - $C_6$  alkyl, as hereinbefore defined,

as well as physiologically tolerated salts and esters thereof.

Peptides which can be obtained from the peptides as claimed in claim 1 by at least



one but at most 10 of the following modifications (a) to (o) of the amino acid chain:

- 5
- (a) the  $\alpha$ -amino acid in position 1 is D-His, Ala, D-Ala, Gly, Lys or D-Lys;
- (b) the  $\alpha$ -amino acid in position 2 is Ser, D-Ser, Thr, D-Thr, Ala, D-Ala, Ile, D-Ile, Val, D-Val, Leu or D-Leu;
- (c) the  $\alpha$ -amino acid in position 3 is D-Glu, D-Asp, Ala or D-Ala;
- (d) the amino acid in position 4 is Ala, D-Ala or  $\beta$ -Ala;
- (e) the  $\alpha$ -amino acid in position 5 is Ser, Tyr or Ala;
- (f) the  $\alpha$ -amino acid in position 6 is Ala, Ile, Val, Leu, Cha or Tyr;
- 10 (g) the  $\alpha$ -amino acid in position 7 is Ala, D-Ala, Tyr, D-Tyr, Ser, D-Ser or D-Thr;
- (h) the  $\alpha$ -amino acid in position 8 is Ala, Tyr or Thr;
- (i) the  $\alpha$ -amino acid in position 9 is Ala, D-Ala, Glu, D-Glu or D-Asp;
- 15 (j) the amino acids in position 10, 11, 12, 15, 16, 17, 18, 19, 20, 21, 24, 28, 29 are, independently of one another, one of the 20 proteinogenic amino acids of Table 1 which are different from the amino acid already present in the respective position in SEQ ID NO: 1 or SEQ ID NO: 2 or a non-proteinogenic D- or L-amino acid;
- (k) the  $\alpha$ -amino acid in position 13 is a neutral L-amino acid apart from Gln;
- 20 (l) the  $\alpha$ -amino acid in position 14 is replaced by a neutral L- or D-amino acid, apart from Met or L-leucine;
- (m) the  $\alpha$ -amino acid in position 22 is D-Phe, Tyr, D-Tyr, Leu, D-Leu, Val, D-Val, L-Cha, D-Cha,  $\beta$ -1-Nal,  $\beta$ -2-Nal or  $\beta$ -1-D-Nal;
- (n) the  $\alpha$ -amino acid in position 23 is Leu, D-Leu, D-Ile, Val, D-Val, L-Cha, D-Cha, Tyr, D-Tyr, Phe or D-Phe; and
- 25 (o)  $\alpha$ -amino acid in position 25, 26 or 27 is a neutral L- or D-amino acid apart from Trp for position 25, Leu for position 26 and Lys for position 27.

3. Peptide according to claim 2 wherein for the modification (a), the amino acid in position 1 is Ala, Gly, or Lys.





4. Peptide according to claim 2 wherein for the modification (b), the  $\alpha$ -amino acid in position 2 is Thr, Ala, Val, Ile or Leu.
5. Peptide according to claim 2 wherein for the modification (c), the  $\alpha$ -amino acid in position 3 is Ala.
6. Peptide according to claim 2 wherein for the modification (d), the  $\alpha$ -amino acid in position 4 is Ala.
- 10 7. Peptide according to claim 2 wherein for the modification (f), the  $\alpha$ -amino acid in position 6 is Ala, Ile, Val, Leu or Tyr.
8. Peptide according to claim 2 wherein for the modification (g), the  $\alpha$ -amino acid in position 7 is Ala, Tyr or Ser.
- 15 9. Peptide according to claim 2 wherein for the modification (i), the  $\alpha$ -amino acid in position 9 is Ala or Glu.
10. Peptide according to claim 2 wherein for the modification (j), the  $\alpha$ -amino acids in position 10, 11, 12, 15, 16, 17, 18, 19, 20, 21, 24, 28, 29 are a proteinogenic L-amino acid.
- 20 11. Peptide according to claim 2 wherein for the modification (k) the  $\alpha$ -amino acid in position 13 is a neutral proteinogenic L-amino acid of Table 1.
12. Peptide according to claim 2 wherein for the modification (l) the  $\alpha$ -amino acid in position 14 is Nle, D-Nle, Ala, D-Ala, Ile, D-Ile, Val or D-Val.
13. Peptide according to claim 12 wherein the  $\alpha$ -amino acid is Ile, Val or Ala.
14. Peptide according to claim 2 wherein for the modification (m) the  $\alpha$ -amino acid in position 22 is Tyr, Leu or Val.



15. Peptide according to claim 2 wherein for the modification (n) the  $\alpha$ -amino acid in position 23 is Leu, Val, Tyr or Phe.
- 5 16. Peptide according to claim 2 wherein for the modification (o) the  $\alpha$ -amino acid in position 25, 26 or 27 is a neutral proteinogenic L-amino acid of Table 1.
17. Peptide as claimed in any one of the claims 1 to 16, wherein the  $\alpha$  amino acid at position 30 is Arg, D-Arg, Tyr or D-Tyr.
- 10 18. Peptide as claimed in claim 17 wherein the  $\alpha$ -amino acid is Arg or Tyr.
19. Peptide as claimed in any one of the claims 1 to 18, wherein it only contains proteinogenic amino acids of Table 1.
- 15 20. Peptide as claimed in any one of the claims 2 to 19, wherein an amino acid substitution has taken place at position 2 compared to sequence 1 or 2.
21. Peptide as claimed in any one of the claims 2 to 20, wherein an amino acid substitution has taken place at position 14 compared to sequence 1 or 2.
- 20 22. Peptide as claimed in any one of the claims 2 to 21, wherein an amino acid substitution has taken place at position 3 compared to sequence 1 or 2.
- 25 23. Peptide, wherein it has one of the sequences 5, 68, 69, 71, 78-82 or 84-91 wherein the N-terminus is represented by  $NR_1R_2$  in which

$R_1$  denotes hydrogen, acetyl, trifluoroacetyl, adamantyl, Fmoc, Z, Boc, Alloc,  $C_1$ - $C_6$  alkyl, as hereinbefore defined,  $C_2$ - $C_8$  alkenyl or  $C_7$ - $C_9$  aralkyl,

$R_2$  denotes hydrogen, acetyl trifluoroacetyl, adamantyl, Fmoc, Z, Boc, Alloc,  $C_4$ - $C_6$  alkyl, as hereinbefore defined,  $C_2$ - $C_8$  alkenyl or  $C_7$ - $C_9$  aralkyl and the



C-terminus is represented by  $COR_3$  in which

$R_3$  equals  $OR_4$  or  $NR_4R_5$

in which  $R_4$  equals hydrogen or  $C_1-C_6$  alkyl as hereinbefore defined

5 in which  $R_5$  equals hydrogen or  $C_1-C_6$  alkyl as hereinbefore defined  
as well as physiologically tolerated salts and esters thereof.

24. Peptide as claimed in any one of the claims 1 to 24, wherein it stimulates the  
release of insulin.

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25. Pharmaceutical preparation containing at least one peptide as claimed in any one of  
the claims 1 to 24 in addition to conventional carriers and auxiliary substances.

26. Use of at least one peptide as claimed in any one of the claims 1 to 24 for the  
15 production of a pharmaceutical composition for the treatment of diabetes.

27. Method of treating diabetes comprising the administration of at least one peptide as  
claimed in any one of claims 1 to 24 to a subject in need thereof.

20 28. Peptide according to claim 1 substantially as hereinbefore described.

29. Pharmaceutical preparation according to claim 7 substantially as hereinbefore  
described.

25 30. Use according to claim 26 substantially as hereinbefore described.



31. Method of treating diabetes substantially as hereinbefore described.

DATED this 30th day of June 2000

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ROCHE DIAGNOSTICS GMBH

by their Patent Attorneys

DAVIES COLLISON CAVE

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